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Protocol Title: A Phase 1/2 Dose-escalation of USL311 as Single Agent and in Combination with Lomustine (CCNU) in Subjects with Advanced Solid Tumors, with Subsequent Single Agent and Combination Phase 2 Cohorts for Subjects with Relapsed/Recurrent Glioblastoma Multiforme (GBM)

Protocol Number: P311-201

Protocol Version: Amendment 4

Issue Date: 02 Aug 2018

Proximagen, LLC
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CLINICAL STUDY PROTOCOL

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Protocol Number:	P311-201
Protocol Version:	Amendment 4
Issue Date:	02 Aug 2018
Clinical Phase:	Phase 1/2
US IND Number:	127616
EudraCT Number:	2015-004214-14
Active Investigational Product:	USL311
Study Population:	Phase 1 Dose-escalation: Subjects with advanced solid tumors for which no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment Phase 2 Dose-expansion: Subjects with relapsed/recurrent GBM who are candidates for re-resection having previously received definitive first line treatment

CONTACT INFORMATION

Sponsor of Clinical Trial:	Proximagen, LLC
Sponsor's Address:	505 Waterford Park Hwy 169 N, Suite 850 Plymouth, MN 55441
Sponsor's Responsible Medical Monitor:	[redacted] MD Proximagen, LLC
Duration of Subject Participation:	Until disease progression, unacceptable toxicity, no longer deriving clinical benefit (in the opinion of the Investigator), or other withdrawal criteria are met. Subjects who discontinue study treatment for reasons other than disease progression will be followed until disease progression or death, or until termination of the study
USL311 IV Starting Dose:	60 mg/m^2
USL311 Oral Starting Dose:	40 mg
Lomustine Starting Dose:	90 mg/m^2

PROTOCOL SYNOPSIS

SPONSOR:

Proximagen, LLC

NAME OF DEVELOPMENT PRODUCT:

USL311

TITLE OF STUDY:

A Phase 1/2 Dose-Escalation of USL311 as Single Agent and in Combination with Lomustine (CCNU) in Subjects with Advanced Solid Tumors, with Subsequent Single Agent and Combination Phase 2 Cohorts for Subjects with Relapsed/Recurrent Glioblastoma Multiforme (GBM)

PROTOCOL NUMBER:

P311-201

STUDY SITES:

Parts 1 and 2 (Phase 1 dose-escalation in advanced solid tumors): approximately 4 study sites. Additional sites may be added depending on study recruitment.

Parts 3 and 4 (Phase 2 dose-expansion in relapsed/recurrent GBM): approximately 10 study sites. Additional sites may be added depending on study recruitment.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

USL311 is initially being evaluated in glioblastoma multiforme (GBM) based on the following:

- Biologic relevance of the target: CXCR4 receptor and its chemokine ligand CXCL12 (SDF-1) have roles in tumor growth and metastasis. CXCR4 is also expressed on most cancer stem cells. CXCR4 antagonism inhibits migration and self-renewal of cancer stem cells while also rendering these cells more sensitive to concurrent therapies.
- Overexpression of CXCR4 appears to be a negative prognostic factor for survival of patients.
- Preclinical data: USL311 and other CXCR4 antagonists inhibit the migration, invasion and proliferation of GBM cell lines, induce apoptosis, and are effective in in vivo orthotopic models. USL311 has demonstrated nonclinical efficacy in both in vitro and in vivo pharmacology models when administered in combination with cytotoxics. Due to its lysosomotropic nature, USL311 may also be an inhibitor of autophagy, and like other autophagy inhibitors is expected to sensitize tumor cells to the effects of cytotoxic agents such as lomustine.
- In contrast to other known CXCR4 antagonists, USL311 crosses the blood-brain barrier.

Initial dose-escalation will be conducted in an advanced solid tumor population for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment. Dose-escalation will be conducted according to a modified continual reassessment method (mCRM) design. Subsequent dose-expansion will allow a more robust investigation of the safety, efficacy, and pharmacokinetics (PK)/pharmacodynamics (PD) of USL311 alone and in combination with lomustine in the target population of relapsed/recurrent GBM. During Phase 2 dose-expansion, subjects will undergo surgical re-resection, as part of their standard-of-care treatment, which will allow for tumor tissue collection and biomarker analysis.

For Part 1a, USL311 will be administered as an intravenous (IV) infusion over 120 minutes (2 hours) once weekly. A weekly dosing schedule was selected because USL311 demonstrates extensive uptake in tissues, including the CNS. Elimination from the tissues is slow, and as the predicted elimination half-life in humans is on the order of days to weeks, target USL311 concentrations may be achieved and maintained with a weekly dosing schedule.

The first-in-human starting dose of weekly IV administration of USL311 will be 60 mg/m^2. The Severely Toxic Dose in 10% of animals (STD 10) was determined as 100 mg/kg USL311 administered as a weekly 2-hour IV infusion in the rat. Based on scaling by body surface area (BSA), the human equivalent dose to the STD 10 is 600 mg/m^2 and thus the starting dose is equivalent to 1/10th of the rat STD 10. This dose is lower than the projected first-in-human starting dose of 267 mg/m^2, calculated as 1/6 of the Highest Non-Severely Toxic Dose (HNSTD) in dogs (80 mg/kg as a weekly 2-hr IV infusion).

As of the date of this amendment, data from all 13 subjects who participated in the Part 1a IV Dose Escalation portion of this study have been collected at USL311 doses of 60, 120, 180, and 250 mg/m^2 via 2- or 4-hour IV infusion. Interim data from the first 8 subjects suggested a potential USL311-related increase in the QT interval, which was transient, resolved post-end of infusion, and exhibited an apparent correlation with plasma USL311 concentrations (i.e., C_{max}). Based on these observations, the duration of USL311 infusion was increased from 120 minutes (2 hours) to 240 minutes (4 hours, i.e., slower infusion rate) and was expected to decrease the magnitude of QT-prolongation (Amendment #3).

Dosing with the 4 hour infusion was initiated at the highest safe dose of USL311 as determined from subjects who received USL311 via a 2 hour infusion (viz., prior to Amendment #3). Subjects who were previously enrolled and were actively participating at the time Protocol Amendment #3 is activated continued study participation according to Protocol Amendment #2.

After 13 subjects were evaluable (i.e., had completed Cycle 1 dosing, at minimum) in Part 1a IV Dose Escalation, the Dose Escalation Committee halted dose escalation, although no DLTs had occurred, due to continuing dose-related increases in QTcF prolongation that was not ameliorated by increasing the infusion duration. Part 1b of the study will resume under this amendment with dosing initiation of an oral formulation of USL311, at a starting dose of 40 mg once daily in 3-week (21-day) cycles. The selected 40 mg daily oral starting dose is comparable to the 30 mg daily starting dose based on 1/6 HNSTD in dogs (5 mg/kg/day oral daily dose) and is lower than the ~60 mg daily dose calculated based on the maximum IV dose tested in humans (250 mg/m^2 weekly, divided into daily doses). USL311 plasma pharmacokinetics are dose-proportional and minimal to no plasma accumulation is expected for USL311 and its main metabolites after daily oral dosing. In addition, a fixed-dosing approach is considered to be appropriate for future studies given that USL311 pharmacokinetics are not influenced by body size. Daily oral dosing is anticipated to further mitigate the concentration-related QT effect, compared to IV bolus weekly dosing.As of the date of this amendment there were no active subjects

currently participating in Part 1a IV Dose Escalation. All subsequent Part 1 participants will be enrolled in Part 1b, Oral Dose Escalation.

Oral USL311 will be used in combination with lomustine during Part 2 of Phase 1 and Part 4 of Phase 2. Lomustine, a cytotoxic drug, is a nitrosourea used in the treatment of certain oncologic conditions and functions as an alkylating agent. Lomustine is commonly used in recurrent GBM and has also been used as the comparator in recent registration-directed trials in this setting. The lomustine starting dose during Part 2 will be 90 mg/m^2. The starting dose was selected based on the anticipated tolerability of the dose based on doses typically used in clinical practice. The maximum lomustine dose allowed during dose-escalation is 130 mg/m^2 which is the maximum dose allowed per the package insert.

OBJECTIVES:

Parts 1 and 2 (Phase 1 Dose-Escalation in Subjects with Advanced Solid Tumors)

The primary objectives of the Phase 1 dose-escalation are to:

- Determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of USL311 as a single agent in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment (Part 1)
- Determine the MTD and RP2D of USL311 in combination with lomustine in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment (Part 2)

The secondary objectives of the Phase 1 dose-escalation are to:

- Assess the safety and tolerability of USL311 as a single agent and in combination with lomustine
- Determine preliminary efficacy parameters such as 6-month progression-free survival rate (PFS-6m), objective response rate (ORR%), disease control rate (DCR), progression free survival (PFS) and overall survival (OS) of USL311 as a single agent and in combination with lomustine
- Determine the PK profile of USL311 in plasma and whole blood and of lomustine in plasma (prior to and with concomitant USL311 administration)
- Evaluate the drug interaction potential between USL311 and lomustine

The exploratory objectives of the Phase 1 dose-escalation are to:

- Determine effects of USL311 on s ystemic markers of CXCR4 inhibition, including measurement of CD34+ cells, and white blood cell (WBC) count
- Measure effects of USL311 on a urine biomarker of phospholipidosis, di-docosahexaenoyl (22:6)-bis(monoacylglycerol) phosphate (BMP)
- Investigate exposure-response relationships for USL311 as a single agent and in combination with lomustine

Parts 3 and 4 (Phase 2 Dose-Expansion in Subjects with Relapsed/Recurrent GBM)

The primary objectives of the Phase 2 dose-expansion are to:

- Determine the percentage PFS-6m of USL311 as a single agent in subjects with relapsed/recurrent GBM who previously received standard-of-care treatment in the first-line setting and who are candidates for re-resection (Part 3)
- Determine the percentage PFS-6m of USL311 in combination with lomustine in subjects with relapsed/recurrent GBM who previously received standard-of-care treatment in the first-line setting and who are candidates for re-resection (Part 4)

The secondary objectives of the Phase 2 dose-expansion are to:

- Assess the safety and tolerability of USL311 as a single agent and in combination with lomustine in subjects with relapsed/recurrent GBM
- Assess ORR%, PFS, DCR and OS of USL311 as a single agent and in combination with lomustine in subjects with relapsed/recurrent GBM
- Determine the PK profile of USL311 in plasma and whole blood and of lomustine in plasma

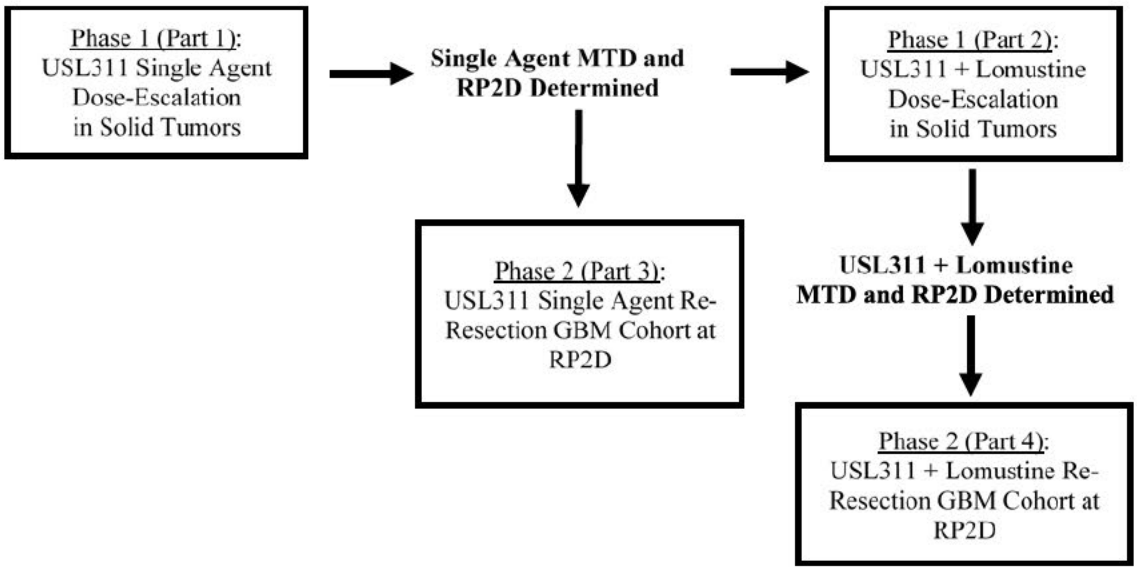
The exploratory objectives of the Phase 2 dose-expansion are to:

- Assess PD markers of CXCR4 inhibition in tumor samples following pre-surgical administration of USL311 for comparison with subjects randomized to receive no pre-surgical USL311 treatment. These markers may include, but are not limited to, the following:
  - Tumor pCXCR4 profile
  - Tumor markers of autophagy (e.g., LC3, Beclin-1)
  - Tumor markers of vasculogenesis (e.g., CD11b) and cancer stem cell survival and proliferation (e.g., nestin, CD133)
- Determine biodistribution of USL311 to the brain/cerebrospinal fluid (CSF) in resected tissue samples and CSF samples following pre-surgical administration of USL311 to investigate CNS penetration and for correlation with clinical response and/or PD data
- Assess exposure-response relationships for USL311 as a single agent and in combination with lomustine
- Assess health-related quality of life (QOL) and subject reported outcomes (European Organization for Research and Treatment of Cancer quality-of-life questionnaire, supplemented with a brain-cancer module [EORTC QLQ-C30/BN20])

STUDY DESIGN:

This is a Phase 1/2 open-label, multicenter study in four parts: Part 1: dose escalation of single-agent USL311 in subjects with advanced solid tumors; Part 2: dose escalation of USL311 in combination with lomustine in subjects with advanced solid tumors; Part 3: preliminary efficacy evaluation of single-agent USL311 in subjects with relapsed/recurrent GBM who are candidates for re-resection, and Part 4: preliminary efficacy evaluation of USL311 in combination with lomustine in subjects with relapsed/recurrent GBM who are candidates for re-resection. Subjects will only participate in one part of the study.

Phase 1/2 Study Components Flow Diagram



Parts 1 and 2 – Phase 1 Dose-Escalation in Subjects with Advanced Solid Tumors:

Dose-escalation components will 1) assess safety, PK/PD and preliminary efficacy of USL311 as a single agent in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment, and 2) assess safety, PK/PD and preliminary efficacy of USL311 in combination with lomustine in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment. The dose-escalation component will determine the MTD and RP2D of single agent USL311 (Part 1) and USL311 in combination with lomustine (Part 2).

Phase 1 dose-escalation for USL311 as a single agent and in combination with lomustine will be conducted according to a mCRM model. Subjects will be enrolled to increasing doses of USL311 (Part 1) or USL311 in combination with lomustine (Part 2). The target DLT (dose limiting toxicity) rate is 33%. The MTD will be defined as the highest safe dose, where safe is defined by having at least a 50% probability that the DLT rate is less than 33%. This definition for safety is consistent with the maximum likelihood estimate for the probability of DLT. If the DLT rate is estimated to be exactly 33%, the probability the DLT rate is less than 33% will be equal to 50%. Therefore, doses with a mean estimated DLT rate less than 33% will be considered safe by this definition and doses with a mean estimated DLT rate greater than 33% will be considered unsafe by this definition.

A dose-toxicity model will be used to determine which doses are expected to be safe and to assign subjects to doses, i.e. to assign subjects to the highest safe dose. However, assignment of dose levels is also governed by rules concerning the speed of dose-escalation and rules that determine whether untried dose levels may be skipped. When the dose is escalated, it may only escalate to the highest safe dose that is no more than a 100% increase over the current dose level. If the dose must be de-escalated, the dose will de-escalate to the highest expected safe dose regardless of how large a decrease in dose level. As long as no DLT has yet been observed, there must be complete DLT information on at least 2 subjects in order to escalate. Once the first DLT is observed in the study (Part 1 and Part 2), there must be complete DLT information on at least 3 subjects in order to escalate. There will be open enrollment to

the study meaning that subjects can be enrolled as they become available for the study, however, generally, there may be no more than 3 subjects enrolled in the study (Part 1 and Part 2) with unknown DLT information at any time.

In the dose-toxicity model, non-DLT toxicity events (i.e., grade 2 toxicities) will inform the probability of DLT at each dose level. To account for the increase in infusion duration from 120 minutes (2 hours) to 240 minutes (4 hours), an additional covariate for infusion duration was included in the dose-toxicity model for Part 1a.

The DLT observation period, for the purpose of dose-escalation, is one cycle. Once a subject has completed this first cycle DLT observation period, the subject will be considered to have complete DLT information for the purposes of making dose-escalation decisions for the next subject enrolling into the study (Part 1 and Part 2). However, DLTs may also appear in later cycles. If a DLT appears at any time through subsequent cycles of treatment, a subject's DLT status and mCRM model will be updated to reflect the late-cycle DLT.

At each dose-escalation point, the Dose Escalation Committee (DEC) will review all of the safety data, any available PK/PD data, and all other relevant subject data in order to recommend the subsequent dose level. Clinical judgment will always supersede the model-based recommendations for dose-escalation, meaning that clinical judgment can be applied to reduce the increment of dose escalation or elect not to dose escalate in favor of accruing more subjects at that dose level.

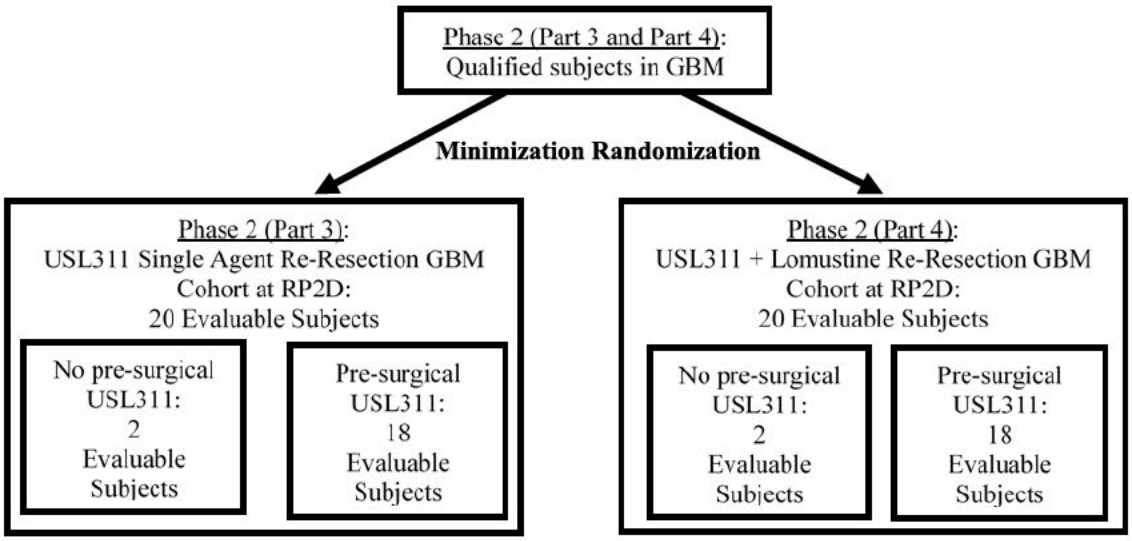
The MTD will be determined by the DEC and Sponsor after considering all available safety, PK, and PD data. Additionally, the RP2D will be determined by the DEC and the Sponsor after considering the safety, PK/PD, and preliminary efficacy outcomes and may be equal to or lower than the MTD.

Parts 3 and 4 – Dose Expansion in Relapsed/Recurrent GBM:

Once the MTDs and RP2Ds of single agent USL311 (Part 1) and USL311 in combination with lomustine (Part 2) have been determined, Phase 2 will assess preliminary efficacy of USL311 (Part 3) and USL311 in combination with lomustine (Part 4) in subjects with relapsed/recurrent GBM, and who are candidates for re-resection. Subjects may begin enrolling into Part 3 after the completion of enrollment in Part 1 and determination of the RP2D for single agent USL311 and subjects may begin enrolling into Part 4 after the completion of enrollment in Part 2 and the determination of the RP2D for USL311 in combination with lomustine. Subjects will be randomized to receive either once daily pre-surgical treatment with USL311 or no USL311 treatment prior to surgery. Subjects randomized to receive pre-surgical treatment with USL311 are required to receive at least one week of treatment prior to surgery, and may receive up to a maximum of three weeks of treatment prior to surgery at the RP2D defined in Parts 1 and 2. The length of treatment will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Exploratory (translational) studies on resected tissue will be performed following surgical resection to determine biodistribution of USL311 in the tumor and to assess PD markers (e.g., pCXCR4 profile, autophagy markers, markers of vasculogenesis and cancer stem cell survival and proliferation). Archiving of tissue samples is recommended to facilitate future biomarker testing. Following surgery, all subjects will initiate treatment of USL311 (Part 3) or USL311 in combination with lomustine (Part 4) within 28 days post-surgery, although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy.

In the Phase 2 dose-expansion, subjects with relapsed/recurrent GBM will be randomized after the Screening visit to one of the four possible treatments shown in the figure below. Due to the potentially staggered start of Parts 3 and 4, a minimization mechanism will be used to assign subjects to Phase 2 treatment in order to obtain a target of 40 response evaluable subjects at the end of the study. Minimization is considered to be an appropriate randomization method for this type of scenario to ensure well-balanced randomization between treatments.

Phase 2 Dose-Expansion in Relapsed/Recurrent GBM: Treatment Allocation



End of Study:

The definition for end of the study is the time of data cutoff for the final analysis or the time of last subject/last treatment, whichever occurs later. The end of the study will not be any earlier than 6 months after the first post-surgical treatment visit of the last subject during Phase 2.

STUDY PLAN:

Plan for Phase 1 IV dose escalation (Part 1a single agent IV USL311):

Treatment will be administered once weekly in 3-week (21-day) cycles. USL311 will be administered IV over 120 minutes (2 hours, Protocol Amendment #2) or 240 minutes (4 hours, Protocol Amendment #3) on Days 1, 8, and 15 of a 21-day cycle.

There are a total of 31 possible dose levels specified between 40 mg/m^2 and 1660 mg/m^2. Dose levels are defined to be in approximately 15% increments.

The dose levels in mg/m^2, are shown below:

USL311 Available IV Doses for Dose-Escalation

Available IV Doses (mg/m^2)			
40	120	320	820
50	140	360	920

60	160	400	1040
70	180	450	1170
80	200	510	1310
90	220	570	1480
100	250	650	1660
110	280	730	

Dose-escalation will begin in the single agent with enrollment of subjects to the 60 mg/m^2 dose. Three subjects will be treated at the initial dose of 60 mg/m^2 and evaluated by the DEC, after all three subjects have completed Cycle 1, before the dose is escalated/de-escalated. The 40 and 50 mg/m^2 dose levels are de-escalation dose levels. For each dose-escalation, one (1) subject will be enrolled at the new dose level and will be treated and observed from Cycle 1-Day 1 through Cycle 1, Day 8 for tolerance to the dose before additional subjects may be treated at that dose level.

Plan for Phase 1 oral dose escalation (Part 1b single agent oral USL311):

Treatment will be administered once daily in 3-week (21-day) cycles. USL311 will be administered as an oral tablet once daily in the morning. It is recommended that subjects take their dose at the same time each day, except for visit days, when the dose will be held and taken during the clinic visit. If a dose is missed in the morning, and fewer than 12 hours have elapsed since the planned administration time, the subject should take the dose. If more than 12 hours have elapsed since the planned administration time, the dose should be skipped.

There are a total of 27 possible dose levels specified between 20 mg and 1440 mg. Dose levels are defined to be in approximately 15% increments, and rounded to the nearest multiple of 20 in order to accommodate dosing with the available 20 mg and 100 mg strengths of the USL311 oral formulation. The dose levels, in mg, are shown below:

USL311 Available Oral Doses for Dose-Escalation

Available Oral Doses (mg)			
20	180	400	1100
40	200	460	1260
60	220	520	1440
80	240	580	
100	260	660	
120	280	740	
140	320	840	
160	360	960	

Dose escalation will begin in the single agent oral formulation with enrollment of subjects to the 40 mg dose. The 20 mg dose level is a de-escalation dose level. For each dose-escalation, one (1) subject will be enrolled at the new dose level and will be treated and observed from Cycle 1-Day 1 through Cycle 1-Day 8 for tolerance to the dose before additional subjects may be treated at that dose level.

Treatment cycles will be repeated every 3 weeks (21 days) until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study. The MTD will be determined for single agent USL311, and all available safety, PK, and PD data will be reviewed by the DEC and Sponsor prior to confirming the optimal starting dosage for USL311 oral administration in combination with lomustine and prior to determining the RP2D. The RP2D of single agent USL311 will be determined by the DEC and Sponsor after considering the safety, PK/PD, and preliminary efficacy outcomes and may be equal to or lower than the MTD.

Plan for Phase 1 dose-escalation (Part 2 USL311 in combination with lomustine):

Once the MTD is determined for oral single agent USL311, dose-escalation of USL311 in combination with lomustine may begin. Lomustine will be administered as a single oral dose every 7 weeks during Cycle 1 and every 6 weeks for subsequent cycles (Cycle 2+) and USL311 will be administered as an oral tablet every day during Cycle 1 starting on Day 8 or over the subsequent 6-week cycles (during Cycle 2+) starting on D ay 1. To evaluate drug-drug interaction potential between USL311 and lomustine, the first dose of lomustine in Cycle 1 will be administered on Cycle 1-Day 1, one week prior to the first dose of USL311 (Day 8). All remaining doses of lomustine (Cycle 2+) will be administered on Day 2 of the cycle. USL311 will be administered daily, continuously as of Cycle 1-Day 8, with no break between cycles.

This dose escalation for USL311 in combination with lomustine will also be conducted according to a mCRM model similar to that for the single agent in Part 1; however, the dose range will be informed by results of USL311 as a single agent. The starting dose of USL311 when combined with lomustine will be at least two (2) evaluated dose levels below the MTD determined for USL311 as a single agent and the starting dose of lomustine will be 90 mg/m^2. A dose de-escalation for lomustine to 80 mg/m^2 may occur depending on the tolerability of the initial lomustine dose. If USL311 is escalated in combination with lomustine to the same dose as the single agent MTD, no further escalation of USL311 will be allowed. However, subsequent subjects may be enrolled to evaluate dose-escalation of lomustine up to a maximum of 130 mg/m^2 in combination with USL311 (dosed at the MTD).

For each dose-escalation, one (1) subject will be enrolled at the new dose level and will be treated and observed from Cycle 1-Day 1 through Cycle 1-Day 15 for tolerance to the combination dose before additional subjects may be treated at the dose level.

Treatment cycles beyond Cycle 1 will be repeated every 6 weeks (42 days) until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study. The MTD will be determined for USL311 in combination with lomustine, and all available safety, PK, and PD data will be reviewed by the DEC and Sponsor prior to determining the RP2D. The RP2D will be determined by the DEC and Sponsor after considering the safety, PK/PD, and preliminary efficacy outcomes and may be equal to or lower than the MTD.

Plan for Phase 2 dose-expansion (Part 3 single agent USL311):

Once the RP2D of single agent USL311 has been determined by the DEC and Sponsor, Phase 2 will assess preliminary efficacy of single agent USL311. Approximately 20 subjects with relapsed/recurrent GBM, who are candidates for re-resection, will be enrolled. Subjects will be randomized to receive once daily pre-surgical treatment with USL311 or no USL311 treatment prior to surgery. Subjects randomized to receive pre-surgical treatment with USL311 are required to receive at least one week of treatment prior to surgery and may receive up to a maximum of three weeks of treatment of USL311 (at

the single agent RP2D defined in Part 1) prior to surgery. The length of treatment will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Following surgery, all subjects (including those randomized to no pre-surgical treatment) will initiate treatment of USL311 within 28 days post-surgery, although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy. USL311 will be administered daily during every 3-week (21-day) cycle, with no breaks between cycles. Treatment cycles will be repeated every 3 weeks (21 days) until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study.

Exploratory (translational) studies on resected tissue will be performed following surgery to determine biodistribution of USL311 in the tumor and assess PD markers (e.g., pCXCR4 profile, autophagy markers, markers of vasculogenesis and cancer stem cell survival and proliferation).

Plan for Phase 2 dose-expansion (Part 4 USL311 in combination with lomustine):

Once the RP2D of USL311 in combination with lomustine has been determined by the DEC and Sponsor, Phase 2 will assess preliminary efficacy of the combination. Approximately 20 subjects with relapsed/recurrent GBM, who are candidates for re-resection, will be enrolled. Subjects will be randomized to receive once daily oral pre-surgical treatment with USL311 or no USL311 treatment prior to surgery. Subjects randomized to receive pre-surgical treatment with USL311 are required to receive at least one week of treatment of USL311 (at the RP2D defined in Part 2) prior to surgery and may receive up to a maximum of three weeks of treatment prior to surgery. The length of treatment received will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Following surgery, all subjects (including those randomized to no pre-surgical treatment) will initiate treatment of USL311 in combination with lomustine within 28 days post-surgery, although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy. USL311 will be administered daily during every 6-week (42-day) cycle, with no breaks between cycles. Lomustine will be administered on Day 2 of every 6-week (42-day) cycle. Treatment cycles will be repeated every 42 days until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study.

Exploratory (translational) studies on resected tissue will be performed following surgery to determine biodistribution of USL311 in the tumor and assess PD markers (e.g., pCXCR4 profile, autophagy markers, markers of vasculogenesis and cancer stem cell survival and proliferation).

NUMBER OF SUBJECTS PLANNED:

The total number of subjects will depend on the number of dose levels assessed in Phase 1 (Parts 1a/1b and Part 2). A total of 13 subjects were enrolled in Part 1a single agent IV dose-escalation. It is expected that approximately 6-40 subjects with advanced solid tumors will be enrolled for Part 1b single agent oral dose-escalation and approximately an additional 6-40 subjects with advanced solid tumors will be enrolled for Part 2 USL311 in combination with lomustine dose-escalation. It is expected that approximately 20 subjects with relapsed/recurrent GBM will be enrolled for each Phase 2 dose-

expansion cohort (Parts 3 and 4), with a total of approximately 40 subjects. The total number of subjects for Phase 1 and 2 combined is expected to be approximately 65-133subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

Inclusion Criteria:

Criteria for both Phase 1 dose-escalation and Phase 2 dose-expansion:

1. Provide signed and dated informed consent prior to study-specific screening procedures
2. ≥18 years old
3. Karnofsky performance score (KPS) ≥ 70
4. Must have adequate bone marrow and renal/hepatic function at the Screening Visit and at Baseline, defined as:
   a. Absolute neutrophil count (ANC) ≥ 1,500/mm^3 without granulocyte colony-stimulating factor (G-CSF) support within 7 days preceding the lab assessment
   b. Platelet count ≥ 100,000/mm^3, without transfusion within 7 days preceding the lab assessment
   c. Hemoglobin (Hgb) ≥ 9 g/dL, without transfusion support within 7 days preceding the lab assessment
   d. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≤ 3 times upper limit of normal (ULN)
   e. Total serum bilirubin ≤ 1.5 times ULN, except in subjects with known Gilbert's Syndrome ≤ 3 times ULN
   f. Serum creatinine ≤ 1.5 times ULN with an estimated creatinine clearance of ≥ 60 mL/min (calculated by the Cockcroft-Gault equation), or creatinine clearance corrected for BSA ≥ 60 mL/min/1.73 m^2 (determined from 24-hour urine collection) per Investigator discretion
   g. Activated partial thromboplastin time/partial thromboplastin time (aPTT/PTT) and prothrombin time (PT) ≤ 1.5 times ULN
   h. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels within the normal range, or above the ULN if considered not clinically significant per the Investigator. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below lower limit of normal must be corrected to within the normal range, or above the ULN if considered not clinically significant per the Investigator, by supplementation prior to starting study drug(s).
5. Disease-free period of > 2 years from any other previous malignancies, excluding curatively treated basal cell carcinoma, squamous cell carcinoma of the skin, or carcinoma *in situ* of the cervix. Subjects with prostate cancer Stage 1 that do not require treatment may also be included.
6. Women of childbearing potential (WOCBP) must have two negative pregnancy tests, the first during Screening and the second within 24 hours prior to first administration of study drug(s) and must agree to use highly effective physician-approved contraception (see Appendix 2) from Screening to 90 days following the last study drug administration. Male subjects must be surgically sterile or must agree to use highly effective physician-approved contraception from Screening to 90 days following the last study drug administration (a barrier method of contraception must be employed by all subjects [male and female], when having sexual intercourse, regardless of other methods).
   a. Females are considered not of childbearing potential if they meet any of the following criteria:
      - Postmenopausal with > 1 year since last menses and:

- If younger than 65 years old, with a follicle-stimulating hormone (FSH) > 40 mIU/mL
- If ≥ 65 years old and not on hormone replacement therapy (HRT), with a FSH > 30 mIU/mL
- If ≥ 65 years old and on HRT, the FSH requirement in not applicable. Postmenopausal females on HRT will be allowed if the treatment is stable for at least 6 months prior to dosing of study drug(s)

- Written medical documentation of being sterilized (e.g. hysterectomy, double oophorectomy, bilateral salpingectomy). Note: Tubal ligation is not considered a form of permanent sterilization.

7. Must be able and willing to comply with the study visit schedule and study procedures
8. Must be able to take oral medications
9. Must have available archived tumor tissue and be willing and able to provide consent for study access to such tissue
10. For subjects with seizures, must be adequately controlled on a stable regimen of anti-epileptic drugs

Criteria for Phase 1 only, dose-escalation in advanced solid tumors:

11. Histologically or cytologically documented diagnosis of a solid tumor for which no standard therapy is recognized or have failed or are intolerant to the standard-of-care treatment
12. Inoperable metastatic or locally advanced, unresectable disease
13. Subjects must have either evaluable or measurable disease
14. Subjects with treated (surgically excised or irradiated) and stable brain metastases are eligible as long as the subject has adequately recovered from treatment and the treatment was ≥ 28 days prior to initiation of study drug(s) and baseline brain computed tomography (CT) with contrast or magnetic resonance imaging (MRI) within 14 days of initiation of study drug(s) is negative for new brain metastases

Criteria for Phase 2 only, dose-expansion in relapsed/recurrent GBM:

15. Histologically confirmed diagnosis of GBM
16. Subjects must have documented recurrence after first-line treatment
17. Prior first-line treatment must have included radiation and temozolomide
18. Subject is suitable for surgical re-resection, per Investigator discretion, as a component of their clinical care
19. Not more than one prior resection (Note: biopsy does not count as prior resection)

Exclusion Criteria:

1. Subjects who have had recent systemic anticancer therapies, interventional device treatment and/or radiotherapy either within 14 days prior to first dose of study drug(s) or have not recovered (to grade ≤ 1) from all clinically significant toxicities related to prior therapies
2. Subjects who have had any major surgery (not including re-resection surgery required in Phase 2) within 28 days prior to first dose of study drug(s), or minor surgery within 14 days prior to first dose of study drug(s)
3. For 14 days prior to first dose of study drugs(s) treatment, administration of any strong cytochrome P450 3A4 (CYP3A4) inducers including, but not limited to, the following:

carbamazepine, ethotoin, mephenytoin, phenobarbital, phenytoin, primidone, rifabutin, rifampin, and St. John's Wort

4. For 14 days prior to first dose of study drug(s) treatment, administration of any strong cytochrome P450 3A4 (CYP3A4) inhibitors including, but not limited to, the following: amprenavir, ataznavir, boceprevir, clarithromycin, conivaptan, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, nefazodone, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, and voriconazole
5. For 14 days prior to first dose of study drug(s) treatment, administration of any agent with moderate-to-high risk to prolong the QTc interval or to cause Torsades de Pointes (see Appendix 3)
6. Subjects who have been treated with an investigational agent or investigational interventional device within 21 days prior to the first dose of study drug(s)
7. Subject is growth factor dependent or transfusion dependent, or has received growth factor support or transfusion support within 14 days prior to the first dose of study drug(s)
8. History of significant cardiac disease. Significant cardiac disease includes the following:
    a. Second/third degree heart block
    b. Significant ischemic heart disease (e.g. myocardial infarction, unstable angina, Grade 3 or 4 [Canadian Cardiovascular Society] angina, hospitalization for ischemic heart disease) within 2 years of first dose of study drug(s)
    c. Family history of long QT syndrome; mean Fridericia corrected QT interval (QTcF) > 450 msec on at least two separate ECGs prior to study start
    d. Poorly controlled hypertension per Investigator opinion
    e. Congestive heart failure of New York Heart Association (NYHA) Class II or worse (slight limitation of physical activity; comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea)
9. Episode of status epilepticus within 1 year prior to the first dose of study drug(s)
10. Pregnant or breastfeeding
11. Any other significant co-morbid conditions that in the opinion of the Investigator would impair study participation or cooperation, including known or suspected history of significant allergic reaction or hypersensitivity to any components of the USL311 formulation

<u>Criteria for Phase 1 only, dose-escalation in advanced solid tumors</u>

12. Subjects with lymphoma as primary cancer

<u>Criteria for Phase 2 only, dose-expansion in relapsed/recurrent GBM:</u>

13. Subjects unable or unwilling to consent to the provision of resected tissue after surgery
14. Prior treatment with plerixafor or another CXCR4 inhibitor
15. Prior treatment with bevacizumab
16. Prior treatment with lomustine and/or carmustine

<u>Criteria for all cohorts receiving oral USL311:</u>

17. Participants with any active medical condition or previous major abdominal surgery or procedure that might, in the investigator's opinion, have a significant effect on USL311 absorption

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

The study drug, USL311, is supplied as a sterile, refrigerated citrate buffered solution, pH 3.5, containing 50 mg/ml of USL311 for IV administration, or as a tablet in 20 mg and 100 mg strengths for oral administration.

The supplied IV solution will be admixed with 250 mL infusion bag of 0.9% Sodium Chloride Injection, USP prior to administration.In Part 1a, USL311 will be administered IV once weekly over a time period of four (4) hours (240 minutes).

In Parts 1b, 2, 3 and 4, USL311 will be administered as oral tablets once daily in the morning under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 1, 2, 8, and 15 and on Cycle 2-Day 1. For all other outpatient dosing days, the subject should be instructed to make an effort to take the oral tablets at least 1 hour before or at least 1 hour after their meal, and they will be instructed to document in their diary the time of any food eaten prior to dosing and for 1 hour after dosing. It is recommended that subjects take their dose at the same time each day, except for visit days when the dose will be held and taken during the clinic visit. If a dose is missed in the morning, and fewer than 12 hours have elapsed since the planned administration time, the subject should take the missed dose. If more than 12 hours have elapsed since the planned administration time, the missed dose should be skipped. On clinic visits where PK will be obtained, the dose will be administered at least 2 hours after the last meal consumed and the subject should not eat for at least 2 hours.

Lomustine will be administered orally once every 6 weeks for all doses, with the exception of Cycle 1 in Part 2, which will be 7 weeks. Lomustine will only be administered during Part 2 and Part 4.

Summary of USL311 dose modification criteria:

Dosing of USL311 may be interrupted to allow for recovery from toxicity, with dosing held for up to 14 days. Thereafter, treatment at the same or a reduced USL311 dose can be considered, based upon discussions between Sponsor or designee and Investigator, if the subject has not developed progressive disease. Subjects with toxicities that require interruptions of greater than 14 days should be discontinued from the study. During Part 2 and Part 4, the combination of USL311 and lomustine will be considered and the most likely agent(s) responsible for the observed toxicity will be modified. Dose modifications should be made based on observed toxicity as follows:

- Grade 1 or 2 toxicity: No requirement for dose interruption or dose reduction. If the toxicity persists at grade 2, a dose reduction to the next lower dose level may be implemented at the discretion of the Investigator.
- Grade 3 toxicity: Dosing should be stopped. USL311 dosing may resume at the next lower dose level when toxicity resolves to grade 1 or returns to baseline.
- Grade 4 toxicity: Dosing should be stopped. USL311 may resume at a lower dose level (1-2 dose level decrease) with the approval of the medical monitor when toxicity resolves to grade 1 or returns to baseline.

Lomustine dose modification criteria per lomustine prescribing information:

Lomustine dose modifications will be based on lomustine prescribing information (see Appendix 4). Doses subsequent to the initial dose should be adjusted according to the hematologic response of the subject to the preceding dose. The following schedule is suggested as a guide to dosage adjustment:

Lomustine Dose Adjustment Guide

Nadir After Prior Dose		Percentage of Prior Dose to be Given
Leukocytes (/mm^3)	Platelets (/mm^3)	
≥4000	≥100,000	100%
3000 – 3999	75,000 – 99,999	100%
2000 – 2999	25,000 – 74,999	70%
<2000	<25,000	50%

A repeat course of lomustine should not be given until circulating blood elements have returned to acceptable levels (platelets above 100,000/mm^3; leukocytes above 4000/mm^3), and this is usually in 6 weeks. Blood counts should be monitored weekly and repeat courses should not be given before 6 weeks because the hematologic toxicity is delayed and cumulative. The use of granulocyte growth factor support will be consistent with the American Society of Clinical Oncology (ASCO) guidelines.

Subjects may be pre-medicated with anti-emetic therapy per ASCO guidelines. Anti-emetic 5-HT3 antagonists with known QTc interval prolongation effects should not be used. Permitted antiemetics are the following: granisetron, palonosetron, aprepitant, fosaprepitant, dexamethasone, and lorazepam.

DURATION OF TREATMENT:

Subjects who tolerate Cycle 1 treatment and do not have progressive disease, treatment failure or relapse are eligible to continue to receive treatment until they experience unacceptable toxicity, disease progression, meet any of the withdrawal criteria, or the study is terminated by the Sponsor.

REFERENCE THERAPIES, DOSE, ROUTE AND REGIMEN:

No reference therapy will be evaluated in this study.

CRITERIA FOR EVALUATION:

DLT:

A DLT will be defined as one or more of the following toxicities occurring during treatment during Phase 1 (Parts 1 and 2) dose-escalation. All toxicities are considered related to study drug (either USL311 and/or lomustine) unless the event is clearly and incontrovertibly due to another cause (e.g., a subject's underlying disease). Toxicities will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03.

Hematologic Toxicity:

- Grade 4 neutropenia lasting > 7 days in the absence of growth factor support
- Grade 3 neutropenia of any duration associated with fever ≥ 38.5°C
- Grade 3 thrombocytopenia with bleeding
- Any other grade 4 hematologic toxicity

Non-hematologic Toxicity:

- Grade 3 nausea, vomiting, and/or diarrhea that lasts > 48 hours despite maximum medical support
- Grade 3 electrolyte imbalance that does not correct within 48 hours to < grade 2 despite maximal medical intervention
- Grade 3 fatigue that does not improve to ≤ grade 2 within 5 days
- Grade 3 QTcF interval prolongation with a ≥ 60 ms change from baseline
  - For DLT assessment based on safety ECGs, baseline is defined as the last ECG collected prior to the first dose of study drug. For DLT assessment based on extracted ECGs, baseline is defined as the closest time-matched extracted ECG (triplicate average) from Cycle 1-Day -1 for Part 1 or the average of the Cycle 1-Day 1 ECG extractions for Part 2
- Any other grade ≥ 3 adverse event (AE) considered at least possibly related to study drug(s), except:
  - Non-hematologic laboratory grade 3 AE that is asymptomatic and/or rapidly reversible (returned to baseline or to grade ≤ 1 within 7 days) unless identified as clinically relevant by the Investigator

Other Toxicities:

- Any IV dose(s) or 7 consecutive oral doses missed within Cycle 1 due to a possible study drug(s)-related toxicity
- Inability to administer scheduled cycle of treatment within 21 days of the scheduled start of Cycle 2 due to a possible study drug(s)-related toxicity

Dose-Escalation Decisions:

A subject must meet one of the following criteria to be evaluable for cohort DLT/MTD/dose-escalation decisions:

- Received all 3 scheduled infusions or missed ≥ 16 of 21 daily oral doses of single agent USL311 during Cycle 1, completed Cycle 1-Day 21, and has not experienced a DLT (Part 1)
- Received all of the 6 scheduled infusions or ≥ 32 of 42 daily oral doses of USL311, as well as the Cycle 1 lomustine dose, has completed Cycle 1-Day 49, and has not experienced a DLT (Part 2)
- Experienced a DLT
- Withdrew from the study prior to completing Cycle 1 due to an AE considered by the investigator to be at least possibly related to study drug(s)

If a subject does not meet any of these criteria, the subject is not evaluable for dose-escalation decisions and will be replaced in that cohort.

Safety:

The following safety outcome measurements will be assessed:

- Incidence of treatment emergent AEs (TEAEs), including DLTs and serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry, hematology, and coagulation assessments)
- Change in vital signs
- Change in ECGs (including Holter monitor extractions and real-time wave form, available bedside on provided equipment)
- Change in physical examination
- Change in neurological examination
- Change in concomitant medication use
- Change in BMP levels (Part 1 and Part 2)

Pharmacokinetics:

In Phases 1 and 2, blood samples will be collected to evaluate the PK of USL311 and its metabolites, as well as lomustine (parent drug and/or active metabolite) PK.

In Phase 2 (and possibly in Phase 1 sub-study), CSF samples will be collected to evaluate the distribution of USL311 to the CNS.

Plasma Sample Collection:

PK samples will be collected according to the schedules in Table 8 – Table 12 and analyzed using a validated liquid chromatography – mass spectrometry (LC-MS) based method. As PK data become available, some samples may become optional if warranted based on ongoing PK data analysis during the study conduct; specific samples and the rationale for making them optional will be documented. Approximately 4 mL of blood will be collected at each nominal time point for the analysis of whole blood (2 mL) and plasma (2 mL) USL311, M10 and 4-AP concentrations. Approximately 4 mL of blood will be collected at each nominal time point for the analysis of plasma lomustine and/or its metabolites concentrations (Parts 2 and 4 only).

CSF Sample Collection:

In Phase 1 (Parts 1 and 2), CSF sample collection is not required. However, if determined to be feasible and appropriate, CSF may be collected as part of a sub-study at select sites and in a subset of subjects. In that sub-study, CSF samples would be collected by lumbar puncture with approximately 1 mL of CSF collected from each subject. Subjects with lumbar puncture contraindications, as determined by the Investigator, would not be eligible for the potential collection of CSF during Parts 1 and 2.

In Phase 2 (Parts 3 and 4), all subjects will have a CSF sample collected during the re-resection surgical procedure. The CSF sample will be collected from the operative field at time of resection. Approximately 1 mL of CSF will be collected from each subject.

Pharmacokinetic Parameters:

Plasma and whole blood PK parameters will be calculated from plasma and whole blood study drug levels, respectively, for USL311, its metabolites, and lomustine and/or its metabolites using non-compartmental and/or compartmental-based methods and will include the following if supported by the available data:

Dose Number	**Pharmacokinetic Parameters**
First Dose	C_{max}, t_{max}, t_{last}, AUC_{0-t}, AUC_{0-tau}, $AUC_{0-\infty}$, AUC % Extrapolated, *CL/F, λ_z, Terminal $t_{1/2}$
Subsequent Doses	C_{max}, t_{max}, C_{min}, t_{min}, C_{avg}, Fluctuation Index (FI), AUC_{0-tau}, *CL_{ss}/F, λ_z, Terminal $t_{1/2}$, V_{ss} (IV only), *V_z/F; Accumulation Ratio and AUC Ratio

* Calculated for USL311 and lomustine (parent drug) only

Additional PK parameters (i.e., area under the moment curve [AUMC], Mean Residence Time, etc.) may be calculated as warranted.

Backup samples may be used for exploratory metabolite profiling of USL311. If conducted, this analysis will be performed independent of the clinical study and described in a separate analysis report.

Biomarker samples:

In Phase 1 (Parts 1 and 2), blood and urine samples will be collected to evaluate biomarkers of target engagement (WBC with differential [neutrophils, lymphocytes, monocytes, eosinophils, and basophils], CD34+ cell counts, and SDF-1 plasma concentration [Part 1a only]) and phospholipidosis (BMP), respectively. Approximately 3 mL 4 mL, and 2 mL of blood will be collected at each nominal time point for WBC count with differential samples, CD34+ count samples, and SDF-1 plasma samples, respectively.

Samples will be collected according to the schedules in Table 8 – Table 12 and analyzed using appropriately validated and/or qualified methods. As data become available, some samples may become optional if warranted based on ongoing analysis during the study conduct; specific samples and the rationale for making them optional will be documented.

In both Phase 1 and Phase 2, optional blood samples may be collected and stored for pharmacogenomics analyses. Samples will only be collected after obtaining consent from the subject with a separate Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved informed consent form (ICF).

Efficacy:

The efficacy outcome of interest will be analysis of PFS-6m following the start of therapy in Part 1 and Part 2 and following the start of therapy post-surgery in Part 3 and Part 4. Secondary efficacy outcomes will also include overall response and overall survival. Progression/response will be determined by the Response Assessment in Neuro-Oncology (RANO) criteria for subjects with primary brain tumors, whereas Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 criteria will be used to determine response for all other subjects.

Exploratory:

The exploratory objectives are as follows:

Phase 1:

- Determine effects of USL311 on systemic markers of CXCR4 inhibition, including measurement of CD34+ cells, and WBC count
- Measure effects of USL311 on a urine biomarker of phospholipidosis BMP
- Investigate exposure-response relationships for USL311 as a single agent and in combination with lomustine

Phase 2:

- Assess PD markers of CXCR4 inhibition in tumor samples following pre-surgical administration of USL311 for comparison with subjects randomized to receive no pre-surgical USL311 treatment. These markers may include, but are not limited to, the following:
  - Tumor pCXCR4 profile
  - Tumor markers of autophagy (e.g., LC3, Beclin-1)
  - Tumor markers of vasculogenesis (e.g., CD11b) and cancer stem cell survival and proliferation (e.g., nestin, CD133)
- Determine biodistribution of USL311 to the brain/CSF in resection tissue sample and CSF sample following pre-surgical administration of USL311 for correlation with clinical response or PD data
- Assess exposure-response relationships for USL311 as a single agent and in combination with lomustine.
- Assess health-related QOL and subject reported outcomes using the EORTC QLQ-C30/BN20

Tissue samples collected for assessment of the exploratory objectives will be analysed using appropriately qualified and/or validated assay methods (i.e., LC-MS/MS, flow cytometry, ELISA).

STATISTICAL METHODS:

Efficacy, safety, PK, PD and QOL assessments will be summarized separately for Phase 1 (Parts 1 and 2; the "dose-escalation cohorts") and Phase 2 (Parts 3 and 4; the "dose-expansion cohorts") of the study. Additional summaries and analyses of pooled Phase 1 and 2 data may also be generated. Descriptive and summary statistics will be presented by dose group and overall as appropriate. Statistics will include number of observations, mean, standard deviation, median, range, and inter-quartile range for continuous variables, and the number and percent for categorical variables; 95% or 90% confidence

intervals will be presented where appropriate. Additional statistics such as geometric mean and coefficient of variation (CV%) may be calculated as warranted.

Analysis Sets:

- Full Analysis Set will include all subjects who received at least one dose of USL311 or lomustine
- Safety Analysis Set will include all subjects who received at least one dose of the USL311 or lomustine and have at least one post-baseline safety evaluation.
- MTD-Evaluable Set will include all subjects in Phase 1 (dose-escalation) who complete one cycle of treatment and assessment or who discontinue before completing the first cycle because of an AE considered by the investigator to be at least possibly related to study drug(s).
- Pharmacokinetic Analysis Set will include all subjects who have received at least one dose of USL311 or lomustine and have at least one quantifiable post-dose blood or plasma concentration of either drug.
- Pharmacodynamic Analysis Sets will include all subjects who have received at least one dose of USL311 or lomustine and have at least one evaluable post treatment assessment for the given PD measure.
- Response-Evaluable Analysis Set will consist of those subjects who have received at least one dose of USL311 or lomustine and have evaluable disease at baseline.
  - In Parts 3 and 4, subjects must have received at least one dose of USL311 or lomustine post-surgery and have at least one non-missing efficacy assessment (i.e., imaging or survival assessment)
- Exposure-Response Analysis Sets will be constructed for each PD or response endpoint to be evaluated for correlation with PK data. The exposure-response analysis sets will consist of all subjects in the PK Analysis Set that also have an evaluable PD/response pretreatment assessment and at least one post-treatment PD/response assessment.

Safety Analyses:

Safety and tolerability will be evaluated in both Phase 1 and Phase 2 components. The Phase 1 primary analyses will include determination of the MTD and RP2D for USL311 as a single agent and in combination with lomustine. All available safety, tolerability, PK and PD data will be considered by the Sponsor in dose-escalation decisions.

In both Phase 1 and Phase 2, the incidence and duration of toxicities will be graded according to the NCI CTCAE version 4.03.

Efficacy Analyses:

Efficacy will be evaluated in both Phase 1 and Phase 2 components. In Phase 1, preliminary efficacy parameters such as PFS-6m, ORR%, DCR, and as defined by RECIST v.1.1 or RANO criteria as appropriate. The Phase 2 analyses will characterize efficacy in subjects in the dose-expansion cohorts treated at the RP2D for USL311 as a single agent and in combination with lomustine as determined by % PFS-6m, PFS, OS, ORR%, DCR, and as defined by RANO criteria.

There is no formal hypothesis testing in this trial for efficacy endpoints. Approximately 20 evaluable subjects will be studied in each of the two Phase 2 dose-expansion groups to provide a preliminary

estimate of efficacy in relapsed/recurrent GBM. The primary objective, PFS-6m, will be calculated with two-sided 90% confidence interval (CI) using Kaplan-Meier (K-M) product-limit estimate of PFS. This will be performed based on both the full analysis set and response evaluable set. Median PFS will be calculated using K-M product-limit estimates and presented with two-sided 90% CIs.

Handling of missing data, drop-outs, outliers and censoring rules will be described in the Statistical Analysis Plan(s) (SAP). Every effort will be made to ensure complete, accurate and timely data collection, and to avoid missing data.

Pharmacodynamic Analyses:

PD data obtained from the exploratory/translational analyses will be summarized using descriptive statistics as appropriate. Correlations between PD parameters and safety and/or efficacy metrics may be explored graphically, and if relationships are observed, exploratory analyses may be performed. Additional exploratory analyses may be conducted and will be described in detail in a separate analysis plan.

Pharmacokinetic Analyses:

PK parameters will be calculated using non-compartmental and/or compartmental methods (such as population PK analysis) as appropriate using actual sample collection times post-dose and will be summarized using descriptive statistics. Mean and individual subject plasma concentration-time profiles for USL311, its metabolites and lomustine and/or its metabolites will be presented on rectilinear and semi-logarithmic scales stratified by dose number and dose level.

Assessments of dose proportionality will be performed using standard methods (i.e., power model approach, population PK analysis) and will be supported by graphical analysis.

Assessment of a PK drug-drug interaction between USL311 and lomustine will be via comparison of USL311 and lomustine PK parameters (AUC, C_{max}) when administered in combination to the PK parameters of each agent when administered individually. Statistical analysis will be performed using standard methods (i.e., ANOVA, non-linear mixed effect modeling) and will be supported by graphical analysis.

Correlations between PK parameters and safety, efficacy and/or PD metrics may be explored graphically and if relationships are observed, exploratory analyses may be performed. Additional exploratory analyses may be conducted and will be described in detail in a separate analysis plan.

Quality of Life/Patient Reported Outcome Measures

In Phase 2, changes in health-related QOL and patient reported outcome measures will be assessed based on the EORTC QLQ-C30/BN20. The QLQ-C30 is a 30-item self-report questionnaire that measures several domains, including physical functioning, role functioning, emotional functioning, cognitive functioning, social functioning, fatigue, pain, nausea and vomiting, and several single items. The BN20 consists of 4 scales comprising multiple items (future uncertainty, visual disorder, motor dysfunction, communication deficit) and 7 single items (headache, seizures, drowsiness, hair loss, itching, difficulty with bladder control, and weakness of both legs).

SCHEDULE OF VISITS AND ASSESSMENTS

Part 1a Schedule of Events (Dose-Escalation Single Agent IV USL311)

Table 1: Dose-Escalation Single Agent IV USL311

Procedures/ Assessements[a]	Screening[b]	Baseline[b,c]	Cycle 1[c]				Cycle 2+[c]		EOT[bb]	Follow-up[bb]	Long-term Follow-up[cc]
	Days -28 to -2	Day -1	Day 1	Day 2	Days 3 & 5	Days 8 & 15	Day 1	Days 8 & 15			
Informed consent[d]	X										
Inclusion/exclusion criteria[b]	X	X									
Medical history[e]	X										
Oncologic history[e,f]	X										
Physical examination[b,g]	X	X				X	X	X	X	X	
Neurological examination[h]	X		X			X	X	X	X	X	
Vital signs[i]	X	X	X			X	X	X	X	X	
Karnofsky performance status	X	X					X Even cycles only		X	X	
Holter-extracted ECG[j]		X	X	X		X	If indicated				
Safety ECG[k]	X		X			X	If indicated		X		
Real-time bedside ECG and heart rate monitoring[l]			X			X	If indicated				
Pregnancy test[m]	X	X					X Even cycles only		X		
Hematology[b,n]	X	X				X	X	X	X	X	
Coagulation assessments[b,o]	X	X				X	X	X	X	X	
Serum chemistry[b,p]	X	X				X	X	X	X	X	
Urinalysis (dipstick)[b,q]	X	X					X		X		

Procedures/ Assessements[a]	Screening[b]	Baseline[b,c]	Cycle 1[c]				Cycle 2+[c]		EOT[bb]	Follow-up[bb]	Long-term Follow-up[cc]
	Days -28 to -2	Day -1	Day 1	Day 2	Days 3 & 5	Days 8 & 15	Day 1	Days 8 & 15			
Cytochrome P450 genotyping/ Pharmacogenomics[r]		X									
Response assessment: RECIST or RANO[s]	X						Cycle 3-Day 1 then every 6 weeks (±7d)		X		
USL311 IV administration[t]			X			X	X	X			
Adverse events[u]	X→	→	→	→	→	→	→	→	→	X	
Concomitant medications[v]	X→	→	→	→	→	→	→	→	→	X	
PK blood sample collection[w,x]			X	X	X	X	X Cycle 2 only				
CD34+ blood sample collection[y]			X	X							
SDF-1 blood sample collection[y]			X	X							
WBC blood sample collection[y]			X	X	X	X	X Cycle 2 only				
BMP urine sample collection[z]		X					X Even cycles only				
Archival tumor tissue sample[aa]	X										
Survival status											X

Footnotes:

[a] All procedures and assessments will be performed before administration of study drug(s), except as indicated below. When multiple assessments are planned at the same time point, ECG assessments (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point) will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal time point.

[b] The following Screening safety assessments are required to be repeated at Baseline (Day -1) if Screening assessments were performed > 3 days before Cycle 1-Day 1 dose: inclusion/exclusion criteria, physical exam, urinalysis (UA), hematology, coagulation, and serum chemistry. If collected ≤ 3 days prior to Cycle 1-Day 1, these assessments do not need to be repeated.

[c] During Cycle 1, the Baseline (Day -1), Day 1 and Day 2 visits will occur on consecutive days; Day 3 and Day 5 PK/PD assessments will be collected according to acceptable PK/PD windows specified in Table 8. Remaining Cycle 1 visits will occur within ≤ 3 days post scheduled study day. All remaining visits (Cycle 2+ up to but not including EOT visit), visits will occur a minimum of 7 days and a maximum of 14 days (unless treatment is on hold for safety reasons) from the previous dose of IV USL311.

[d] The IRB/IEC-approved ICF must be signed before any study-specific procedures or examinations are performed.

[e] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until first dose of study drug will be recorded as an AE.

[f] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[g] Physical exams at Screening, Baseline (Day -1), Day 1 of Cycle 2+, and at Follow-up should be complete assessments. Other physical exams may be abbreviated, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit; height at Screening only.

[h] Neurological exams at Screening and Follow-up will be complete assessments. At Cycle 1-Day 1, complete neurological exams will be performed pre-dose and prior to clinic discharge (between 5 hr nominaltime point and discharge); abbreviated neurological exams will be performed at 2 hr and EOI. On Cycle 1-Day 8 and Day 15, neurological exams will be performed pre-dose and at EOI and may be abbreviated exams. Other scheduled neurological exams will be performed pre-dose only, if applicable, and may be abbreviated exams, at the Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Neurological exams will be performed within 0.5 hr (mid-infusion) and ≤ 1 hr (EOI) after the nominal time point unless otherwise stated.

[i] On visits without study drug administration, vital signs will be collected once. On days of study drug administration during all cycles, vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to dosing and prior to clinic discharge. Additionally, at Cycle 1 only, vital signs will be collected 2 hr post start of infusion and EOI. Subjects should be in semi-recumbent position for ≥ 5 mins prior to collection. Vital signs will be collected ± 10 mins relative to nominal time point; when multiple assessments are scheduled at the same nominal time point, collect vital signs following ECG extraction period (see footnote j), prior to PK sample collection. Additional vital signs assessments should be performed if clinically indicated.

[j] Holter-extracted 12-lead ECGs to be performed on Day -1 and Cycle 1-Day 1 for approximately 8.5 hours occurring at equivalent time points (± 30 mins) on both days. On Cycle 1-Day 1, ECG extractions will occur at the followingnominal time points: 30 mins predose, and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hours post-start of infusion. ECGs will also be extracted on Cycle 1-Day 2 at 24 hours post start of Day 1 infusion; on Cycle 1-Day 8 pre-dose, and 0.5, 1.5, 3, and 4 hours post start of infusion; and on Cycle 1-Day 15 pre-dose, 2 and 4 hours post start of infusion. Subjects will rest in semi-recumbent position for at least 20 minutes per timepoint: from ≥ 5 minutes prior to ECG collection period which spans from -15 to -5 minutes prior to the nominal time point No other assessments will be performed during ECG collection period except for safety ECG collections. Frequency of ECGs may be increased, and/or included at subsequent cycles if clinically indicated.

[k] Safety ECGs will be collected (via ECG printouts) by clinic staff at Screening and EOT visits in triplicate with replicates at least 2 minutes apart. Single (i.e., non-triplicate) safety ECGs will also be collected during Cycle 1 on Days 1, 8 and 15 at the following nominaltime points: Predose, and 0.5, 1, 1.5, 2, 3 and 4 hours post start of infusion. Subjects will rest in semi-recumbent position for ≥ 5 minutes prior to safety ECG collection and will remain in this position throughout ECG collection. When safety ECGs are to be collected at the same nominal time as an ECG extraction, the safety ECGs should be collected during the ECG extraction period which spans from -15 minutes to -5 minutes prior to the nominal time point (refer to footnote j). Additional safety ECGs may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Frequency of safety ECGs may be increased and/or included at subsequent cycles if clinically indicated.

[l] Real-time bedside ECGs and heart rate monitoring will be performed on provided equipment during Cycle 1 on Days 1, 8 and 15 from start of infusion through clinic discharge (approximately 6 hours post start of infusion). Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-time bedside monitoring will be performed at subsequent cycles as clinically indicated.

[m] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed at Baseline (Day -1) of Cycle 1 and pre-dose on Day 1 of every even-numbered cycle (i.e., Cycle 2-Day 1; Cycle 4-Day 1; Cycle 6-Day 1, etc.), and at the EOT visit. If treatment with study drug is stopped for ≥14 days, subject should have a negative pregnancy test prior to restarting study drug.

[n] Hematology performed weekly by local laboratory: red blood cell (RBC) count, Hgb, hematocrit (Hct), mean corpuscular volume (MCV), platelets, WBC count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), absolute lymphocyte count (ALC), and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring as clinically appropriate. Hematology assessments scheduled for day of dosing must be available and assessed for toxicity before dosing. Hematology sample can be drawn ≤ 24 hours prior to dosing.

[o] Coagulation assessments performed weekly by local laboratory: prothrombin time (PT), activated partial thromboplastin time (aPTT)/PTT, and international normalized ratio (INR). Coagulation assessments scheduled for day dosing must be available and assessed for toxicity before dosing. Coagulation sample can be drawn ≤ 24 hours prior to dosing.

[p] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, alkaline phosphatase (ALP), lactic dehydrogenase (LDH), AST, ALT, glucose, bicarbonate, blood urea nitrogen (BUN), uric acid, creatine phosphokinase (CPK) and gamma-glutamyltransferase (GGT). Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below lower limit of normal (LLN) must be corrected to within normal range, or above the ULN if deemed not clinically significant by the Investigator, prior to study drug dosing. Serum chemistry assessments scheduled for day of dosing must be available and assessed for toxicity before dosing. Serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[q] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up on clinically significant urine dipstick findings, as determined by the investiag.

[r] A blood sample for cytochrome P450 genotyping will be collected on Baseline (Day -1). An optional blood sample for future pharmacogenomics analyses may also be collected on Day -1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[s] Tumor assessments to be performed at Screening, Cycle 3-Day 1, then every 6 weeks (± 7 days) thereafter and at EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using same techniques. RECIST v.1.1 will be used to determine response, with exception of RANO criteria for primary brain tumor assessment.

[t] It is recommended that, when possible, subjects should remain in semi-recumbent position throughout the duration of USL311 infusion.

[u] The AE/SAE collection period is from ICF signing through the Follow-up Visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up Visit should be followed to resolution, until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[v] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose until the Follow-up Visit. For primary brain tumor and GBM subjects, corticosteroid use, including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[w] Blood samples (approximately 4 mL) for PK analysis (USL311) will be collected on Cycle 1-Day 1, Day 2, Day 3, Day 5, Day 8, and Day 15 and a pre-dose sample prior to the first dose of Cycle 2.

[x] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[y] Blood samples (approximately 2 or 6 mL each) for PD analyses (CD34+ [6 mL], SDF-1 [2 mL], and WBC count with differential [neutrophils, lymphocytes, monocytes, eosinophils, and basophils] [2 mL]) will be collected on Cycle 1-Day 1 at predose, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours post-dose, on Day 2 at 24 hours post the Day 1 dose, Day 3, Day 5, Day 8 at predose, 0.5, 1.5, 3, and 4 hours post-dose, and Day 15 at predose and 2 and 4 hours post-dose and on Cycle 2-Day 1 a pre-dose sample prior to the first dose of Cycle 2 as described in Table 8.

[z] BMP urine sample collection to occur on Baseline (Day -1) and prior to Day 1 dosing on even-numbered cycles (i.e., Cycle 2-Day 1; Cycle 4-Day 1; Cycle 6-Day 1; etc.).

[aa] Confirmation of archival tumor tissue availability is required prior to enrollment.

[bb] The EOT visit will occur within ≤ 14 days of the decision to discontinue study treatment; the Follow-up Visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up Visit; however, the Follow-up Visit should occur as close as possible to 28 days after the last USL311 dose. Any TEAEs ongoing at the EOT Visit should be followed to resolution or until the Investigator considers them chronic or stable.

[cc] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival follow-up information will be collected via telephone calls and/or clinic visits.

Part 1b Schedule of Events (Dose-Escalation Single Agent Oral USL311)

Table 2: Dose-Escalation Single Agent Oral USL311

Procedures/ Assessements[a]	Screening[b]	Baseline[b,c]	Cycle 1[c]			Cycle 2+[c]		EOT[bb]	Follow-up[bb]	Long-term Follow-up[cc]
	Days -28 to -2	Day -1	Day 1	Day 2	Days 8 & 15	Day 1	Days 8 & 15			
Informed consent[d]	X									
Inclusion/exclusion criteria[b]	X	X								
Medical history[e]	X									
Oncologic history[e,f]	X									
Physical examination[b,g]	X	X			X	X	X	X	X	
Neurological examination[h]	X		X		X	X	X	X	X	
Vital signs[i]	X	X	X		X	X	X	X	X	
Karnofsky performance status	X	X				Even cycles only		X	X	
Holter-extracted ECG[j]		X	X	X	X	X	If indicated			
Safety ECG[k]	X		X	X	X	X	If indicated	X		
Real-time bedside ECG and heart rate monitoring[l]			X		X	If indicated				
Pregnancy test[m]	X	X				Even cycles only		X		
Hematology[b,n]	X	X			X	X	X	X	X	
Coagulation assessments[b,o]	X	X			X	X	X	X	X	
Serum chemistry[b,p]	X	X			X	X	X	X	X	
Urinalysis (dipstick)[b,q]	X	X				X		X		

Procedures/ Assessements[a]	Screening[b]	Baseline[b,c]	Cycle 1[c]			Cycle 2+[c]		EOT[bb]	Follow-up[bb]	Long-term Follow-up[cc]
	Days -28 to -2	Day -1	Day 1	Day 2	Days 8 & 15	Day 1	Days 8 & 15			
Cytochrome P450 genotyping/ Pharmacogenomics[r]		X								
Response assessment: RECIST or RANO[s]	X					Cycle 3-Day 1 then every 6 weeks (± 7d) thereafter		X		
USL311 oral administration[t]			X→	→	→	→	→			
Adverse events[u]	X→	→	→	→	→	→	→	→	X	
Concomitant medications[v]	X→	→	→	→	→	→	→	→	X	
PK blood sample collection[w,x]			X	X	X	X Cycle 2 only				
CD34+ blood sample collection[y]			X	X						
WBC blood sample collection[y]			X	X	X	X Cycle 2 only				
BMP urine sample collection[z]		X				X Even cycles only				
Archival tumor tissue sample[aa]	X									
Survival status										X

Footnotes:

[a] All procedures and assessments will be performed before the administration of study drug(s), except as indicated below. When multiple assessments are planned at the same time point, ECG assessments (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point), will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal (scheduled) time point.

[b] The following Screening safety assessments are required to be repeated at Baseline (Day -1) if Screening assessments were performed > 3 days before Cycle 1-Day 1 dose: inclusion/exclusion criteria, physical exam, UA, hematology, coagulation and serum chemistry. If collected ≤ 3 days prior to Cycle 1-Day 1, these assessments do not need to be repeated.

[c] During Cycle 1, the Baseline (Day -1), Day 1 and Day 2 visits will occur on consecutive days; Day 3 and Day 5 PK/PD assessments will be collected according to the acceptable windows specified in Table 9. Remaining Cycle 1 visits will occur within ≤ 3 days post scheduled study day. All remaining visits (Cycle 2+ up to but not including EOT visit), will occur within ± 3 days of scheduled visit.

[d] IRB/IEC-approved ICF must be signed before any study-specific procedures or examinations are performed.

[e] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until first dose of study drug will be recorded as an AE.

[f] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[g] Physical exams at Screening, Baseline (Day -1), Day 1 of Cycle 2+, and Follow-up should be complete assessments. Other physical exams may be abbreviated, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit; height at Screening only.

[h] Neurological exams at Screening and Follow-up will be complete assessments. At Cycle 1-Day 1, complete neurologic exams will be performed pre-dose and > 4 hours after dosing, prior to discharge. At Cycle 1-Day 8 and Day 15, neurological exams will be performed pre-dose and may be abbreviated exams. Other scheduled neurological exams will be performed pre-dose, if applicable, and may be abbreviated exams, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms.

[i] Vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to in-clinic dosing, and prior to clinic discharge. If PK collection is prior to vitals, wait ≥ 5 minutes before obtaining vitals. Subjects should be in semi-recumbent position for ≥ 5 mins prior to collection. Perform additional vital signs if indicated.

[j] Holter-extracted 12-lead ECGs will be performed on Day -1 and Cycle 1-Day 1 for approximately 8.5 hours, occurring at equivalent time points (± 30 mins) on both days. On Cycle 1-Day 1, ECG extractions will occur at the following time points: Predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, and 6 hours post-dose. ECGs will also be extracted on Cycle 1-Day 2 at 24 hours post Cycle 1-Day 1 dose (prior to Day 2 dose); on Cycle 1-Day 8 pre-dose, and 0.5, 2, 3 and 4 hours post-dose; on Cycle 1-Day 15 pre-dose, and 2-4 hours post-dose (to be aligned with the PK collection time) and on Cycle 2-Day 1 pre-dose and 2-4 hours post-dose (to be aligned with the PK collection time. The holter device will be placed at least 30 minutes prior to dosing and the subject will be placed in a resting, semi-recumbent position and will remain in a semi-recumbent position for at least 4 hours post-dose. No other assessments will be performed during each ECG collection period (i.e., from -15 to -5 minutes prior to the nominal time point) except for safety ECG collections. Frequency of ECGs may be increased and/or included at subsequent cycles if clinically indicated. All other electrical devices need to be removed from the bed/chair including cell phones.

[k] Safety ECGs will be collected (via ECG printouts) by clinic staff at Screening and EOT visits in triplicate with replicates at least 2 minutes apart. Single (i.e., non-triplicate) safety ECGs will also be collected during Cycle 1-Day 1 predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, and 6 hours post-dose; on Cycle 1-Day 2 at 24 hours post Cycle 1-Day 1 dose (prior to Day 2 dose); on Cycle 1-Day 8 pre-dose, and 0.5, 2, 3 and 4 hours post-dose; on Cycle 1-Day 15 pre-dose, and 2-4 hours post-dose (to be aligned with the PK collection time) and on Cycle 2-Day 1 pre-dose and 2-4 hours post-dose (to be aligned with the PK collection time). Subjects will rest in semi-recumbent position for ≥ 5 minutes prior to safety ECG collection and will remain in this position throughout ECG collection. When safety ECGs are to be collected at the same nominal time point as a Holter-extracted ECG, the safety ECGs should be collected during the Holter ECG extraction period, which spans from -15 minutes to -5 minutes prior to the nominal time point (refer to footnote j). Additional safety ECGs may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Frequency of safety ECGs may be increased and/or included at subsequent cycles if clinically indicated.

[l] Real-time bedside ECGs and heart rate monitoring will be performed on provided equipment during Cycle 1 on Days 1, 8 and 15 after in-clinic dosing. Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-time bedside monitoring will be performed at subsequent cycles as clinically indicated.

[m] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed Baseline (Day -1) of Cycle 1 and pre-dose on Day 1 of every even-numbered cycle (i.e., Cycle 2-Day 1; Cycle 4-Day 1; Cycle 6-Day 1, etc.), and at the EOT visit. If treatment with study drug is stopped for ≥14 days, subject should have a negative pregnancy test prior to restarting study drug.

[n] Hematology performed weekly by local laboratory: RBC count, Hgb, Hct, MCV, platelets, WBC count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), ALC, and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring, as clinically appropriate. Hematology sample can be drawn ≤ 24 hours prior to visit.

[o] Coagulation performed weekly by local laboratory: PT, aPTT/PTT, and INR. Coagulation sample can be drawn ≤ 24 hours prior to visit.

[p] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, ALP, LDH, AST, ALT, glucose, bicarbonate, BUN, uric acid, CPK and GGT. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below LLN must be corrected to within the normal range, or above the ULN if deemed not clinically significant by the Investigator, prior to dosing. Serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[q] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up on clinically significant urine dipstick findings, which include a positive result for protein, blood, nitrite, and/or leukocyte esterase.

[r] A blood sample for cytochrome P450 genotyping will be collected on Baseline (Day -1). An optional blood sample for future pharmacogenomics analyses may also be collected on Day -1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[s] Tumor assessments to be performed at Screening, Cycle 3-Day 1, then every 6 weeks (± 7 days) thereafter and at EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using the same techniques. RECIST v.1.1 will be used to determine response, with exception of RANO criteria for primary brain tumor assessment. A confirmed objective response is defined as a response that persists on repeat imaging for two assessments with a time period of at least 4 weeks between the two assessments. In the event of a confirmed response, the timing of subsequent tumor assessment will be reset at the 6-week (± 7 days) interval from confirmatory scan.

[t] Study drug will be held on the morning of clinic visits and will be administered in-clinic under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 1, 2, 8 and 15 and on Cycle 2-Day 1, after pre-dose assessments have been completed.

[u] The AE/SAE collection period is from ICF signing through the Follow-up visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up visit should be followed to resolution, until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[v] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose until the Follow-up visit. For primary brain tumor and GBM subjects, corticosteroid use, including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[w] Blood samples (approximately 4 mL) for PK analyses (USL311) will be collected on Cycle 1-Day 1, Day 2, Day 8, and Day 15, and on Cycle 2-Day 1. Collection schedule and acceptable PK sample collection windows are as defined in Table 9.

[x] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[y] Blood samples (approximately 2 or 6 mL each) for PD analyses (CD34+ [6 mL], and WBC count with differential [neutrophils, lymphocytes, monocytes, eosinophils, and basophils] [2 mL]) will be collected on Cycle 1-Day 1, Day 2, Day 8, and Day 15. Collection schedule and acceptable PD sample collection windows are defined in Table 9.

[z] BMP urine sample collection to occur on Baseline (Day -1) and prior to dosing on Day 1 of every even-numbered cycle (i.e., Cycle 2-Day 1; Cycle 4-Day 1; Cycle 6-Day 1; etc.).

[aa] Confirmation of archival tumor tissue availability is required prior to enrollment.

[bb] The EOT visit will occur within ≤ 14 days of the decision to discontinue study treatment; the Follow-up visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up visit; however, the Follow-up visit should occur as close as possible to 28 days after the last USL311 dose. Any TEAEs ongoing at the EOT visit should be followed to resolution or until the Investigator considers them chronic or stable.

[cc] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival follow-up information will be collected via telephone calls and/or clinic visits.

Part 2 Schedule of Events (Dose-Escalation USL311 + Lomustine)

Table 3: Dose-Escalation Oral USL311 + Lomustine

Procedures/ Assessments[a]	Screening[b]	Cycle 1[c, d]					Cycle 2+[c, d]			EOT[cc]	Follow-up[cc]	Long-term Follow-up[dd]
	Days -28 to -1	Day 1	Day 8	Day 9	Days 15 & 22	Days 29, 36 & 43	Day 1	Day 2	Days 8, 15, 22, 29, 36			
Informed consent[e]	X											
Inclusion/exclusion criteria[b]	X	X										
Medical history[f]	X											
Oncologic history[f,g]	X											
Physical examination[b,h]	X	X	X		X	X	X		X	X	X	
Neurological examination[i]	X		X		X	X	X		X	X	X	
Vital signs[j]	X	X	X		X	X	X		X	X	X	
Karnofsky performance status	X	X					X			X	X	
Holter-extracted ECG[k]		X	X	X	X	X	X	If indicated				
Safety ECG[l]	X	X	X	X	X	X	X	If indicated		X		
Real-time bedside ECG and heart rate monitoring[m]			X		X	X	If indicated					
Pregnancy test[n]	X	X					X			X		
Hematology[b,o]	X	X	X		X	X	X		X	X	X	
Coagulation assessments[b,p]	X	X	X		X	X	X		X	X	X	
Serum chemistry[b,q]	X	X	X		X	X	X		X	X	X	
Urinalysis (dipstick)[b,r]	X	X					X			X		
Cytochrome P450 genotyping/ Pharmacogenomics[s]		X										

Procedures/ Assessments[a]	Screening[b]	Cycle 1[c, d]					Cycle 2+[c, d]			EOT[cc]	Follow-up[cc]	Long-term Follow-up[dd]
	Days -28 to -1	Day 1	Day 8	Day 9	Days 15 & 22	Days 29, 36 & 43	Day 1	Day 2	Days 8, 15, 22, 29, 36			
Response assessment: RECIST or RANO[t]	X						At Cycle 2-Day 1 then every 6 weeks (± 7d) thereafter			X		
USL311 oral administration[d,u]			X→	→	→	→	→	→	→			
Lomustine administration[d]		X						X				
Adverse events[v]	X→	→	→	→	→	→	→	→	→	→	X	
Concomitant medications[w]	X→	→	→	→	→	→	→	→	→	→	X	
USL311 PK blood sample collection[x,y]			X	X	X		X Cycle 2 only	X Cycle 2 only				
Lomustine PK blood sample collection[x,y]		X						X Cycle 2 only				
CD34+ blood sample collection[z]			X	X	X							
WBC blood sample collection[z]			X	X	X		X Cycle 2 only					
BMP urine sample collection[aa]		X					X					
Archival tumor tissue[bb]	X											
Survival status												X

Footnotes:

[a] All procedures and assessments will be performed before the administration of study drug, except as indicated below. When multiple assessments are planned at the same time point, ECG assessment (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point), will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal (scheduled) time point.

[b] The following Screening safety assessments are required to be repeated at Cycle 1-Day 1 (baseline) if Screening assessments were performed > 3 days before Cycle 1-Day 1 dose (1st dose of lomustine): inclusion/exclusion criteria, physical exam, UA, hematology, coagulation and serum chemistry. If collected ≤ 3 days prior to Cycle 1-Day 1, these assessments do not need to be repeated.

[c] During Cycle 1, the Day 8 and Day 9 visits will occur on consecutive days. All remaining Cycle 1 visits will occur on the scheduled study day or ≤ 3 days post scheduled study day. During Cycle 2-Day 1 and Day 2 visits will occur on consecutive days. All remaining visits (Cycle 2+ up to but not including the EOT visit) will occur within ± 3 days of scheduled visit.

[d] In Cycle 1 only, the cycle duration is 7 weeks (Day 1 – Day 49); lomustine will be administered on Cycle 1-Day 1 and the first dose of USL311 will be administered on Cycle 1-Day 8. In all subsequent cycles, the cycle duration is 6 weeks (Day 1 – Day 42); USL311 will be administered beginning on Day 1 and lomustine will be administered on Day 2 of each cycle.

[e] The IRB/IEC-approved ICF must be signed before any study-specific procedures or examinations are performed.

[f] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until first dose of study drug will be recorded as an AE.

[g] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[h] Physical exams at Screening, Day 1 of Cycle 2+, and Follow-up should be complete assessments. Other physical exams may be abbreviated, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit, height at Screening only.

[i] Neurological exams at Screening and Follow-up will be complete assessments. At Cycle 1-Day 8, complete neurological exams will be performed pre-dose and > 4 hours after dosing, prior to clinic discharge. At Cycle 1-Day 15, Day 22, Day 29, Day 36, and Day 43, neurological exams will be performed pre-dose and may be abbreviated exams. Other scheduled neurological exams will be performed prior to in-clinic dosing only, and may be abbreviated exams, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms.

[j] Vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to in-clinic USL311 dosing, and prior to clinic discharge. If PK collection is prior to vitals, wait ≥ 5 minutes before obtaining vitals. Subjects should be in a semi-recumbent position for ≥ 5 mins prior to collection. Perform additional vital signs if clinically indicated.

[k] Holter-extracted 12-lead ECGs will be performed on Cycle 1-Day 1 at -60 min, -45 min, and -30 min prior to lomustine dosing; on Cycle 1-Day 8 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, and 6 hours post-dose. ECGs will also be extracted on Cycle 1-Day 9 at 24 hours post Cycle 1-Day 1 dose (prior to Day 2 dose); on Cycle 1-Day 15 pre-dose, and 0.5, 2, 3 and 4 hours post-dose; on Cycle 1-Day 22 pre-dose, and 2-4 hours post-dose (to be aligned with the PK collection time) and on Cycle 1-Days 29, 36, and 43 prior to in-clinic dosing with USL311 and 2 hours post-dose. ECGs will also be extracted on Cycle 2-Day 1 pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 6 hours post-dose and on Cycle 2-Day 2 at 24 hours post Cycle 2-Day 1 dose (prior to Day 2 dose), and 1, 2, 3, 4, 5, 8 and 12 hours post-dose. On Cycle 1-Day 1, subjects will rest in a semi-recumbent position from -80 min (i.e., 20 minutes prior to the -60 min time point) through -20 min prior to lomustine administration. On all other days, the holter device will be placed at least 30 minutes prior to dosing and the subject will be placed in a resting, semi-recumbent position and will remain in a semi-recumbent position for at least 4 hours post-dose. No other assessments will be performed during each ECG collection period (i.e., from -15 to -5 minutes prior to the nominal time point) except for safety ECG collections. Frequency of ECGs may be increased and/or included at subsequent cycles if clinically indicated. All other electrical devices need to be removed from the bed/chair including cell phones.

[l] Safety ECGs will be collected (via ECG printouts) by clinic staff at Screening and EOT visits in triplicate with replicates at least 2 minutes apart. Single (i.e., non-triplicate) safety ECGs will also be collected during Cycle 1 as follows: on Cycle 1-Day 8 predose and 0.5, 1, 1.5, 2, 2.5, 3, 4 and 6 hours post-dose; Day 9 at 24 hours post-dose (prior to in-clinic dosing); Day 15 pre-dose and 0.5, 2, 3, and 4 hours post-dose; Day 22 pre-dose and 2-4 hours post-dose (to be aligned with the PK collection time) and Cycle 1-Day 29, Day 36 and Day 43 pre-dose and 2 hours post-dose. Subjects will rest in semi-recumbent position for ≥ 5 minutes prior to safety ECG collection and will remain in this position throughout ECG collection. When safety ECGs are to be collected at the same nominal time as a Holter-extracted ECG, the safety ECGs should be collected during the Holter ECG extraction period, which spans from -15 minutes to -5 minutes prior to the nominal time point (refer to footnote k). Additional safety ECGs may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Frequency of safety ECGs may be increased and/or included at subsequent cycles if clinically indicated.

[m] Real-time bedside ECGs and heart rate monitoring will be performed on provided equipment during Cycle 1 on Days 8, 15, 22, 29, 36 and 43 in-clinic dosing. Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-tine bedside monitoring will be performed at subsequent cycles as clinically indicated.

[n] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed prior to dosing with lomustine on Cycle 1-Day 1, prior to USL311 dosing on Day 1 of every cycle thereafter, and at the EOT visit. If treatment with study drug is stopped for ≥14 days, subject should have a negative pregnancy test prior to restarting study drug.

[o] Hematology performed weekly by local laboratory: RBC count, Hgb, Hct, MCV, platelets, WBC count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), ALC, and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring, as clinically appropriate. Hematology sample can be drawn ≤ 24 hours prior to visit.

[p] Coagulation assessments performed weekly by local laboratory: PT, aPTT/PTT, and INR. Coagulation sample can be drawn ≤ 24 hours prior to dosing.

[q] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, ALP, LDH, AST, ALT, glucose, bicarbonate, BUN, uric acid, CPK, and GGT. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below LLN must be corrected to within the normal range, or above the ULN if deemed not clinically significant by the Investigator The serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[r] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up on clinically significant urine dipstick findings, which include a positive test for protein, blood, nitrite and/or leukocyte esterase.

[s] A blood sample for cytochrome P450 genotyping will be collected on Cycle 1-Day 1. An optional blood sample for future pharmacogenomics analyses may also be collected on Cycle 1-Day 1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[t] Tumor assessments to be performed at Screening, Cycle 2-Day 1, then every 6 weeks (± 7 days) thereafter and at EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using same techniques. RECIST v1.1 will be used to determine response with exception of RANO criteria for primary brain tumor assessment. A confirmed objective response is defined as a response that persists on repeat imaging for two assessments with a time period of at least 4 weeks between the two assessments. In the event of a confirmed response, the timing of subsequent tumor assessment will be reset at the 6-week (± 7 days) interval from confirmatory scan.

[u] Study drug will be held on the morning of clinic visits and will be administered in-clinic under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 8, 9, 15 and 22 and on Cycle 2-Days 1 and 2, after pre-dose assessments have been completed. [v] The AE/SAE collection period is from ICF signing through the Follow-up visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up visit should be followed to resolution or until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[w] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose of lomustine until the Follow-up visit. For primary brain tumor subjects, corticosteroid use including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[x] Blood samples (approximately 4 mL) for PK analyses (USL311 and/or lomustine) will be collected on Cycle 1-Day 1, Day 8, Day 9, Day 15 and Day 22, and on Cycle 2-Day 1 and Day 2. Collection schedule and acceptable PK sample collection windows are as defined in Table 10.

[y] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[z] Blood samples (approximately 2 or 6 mL each) for PD analyses (CD34+ [6 mL], and WBC count with differential [neutrophils, lymphocytes, monocytes, eosinophils, and basophils] [2mL]) will be collected on Cycle 1-Day 8, Day 9, Day 15, and Day 22, and Cycle 2-Day 1. Collection schedule and acceptable PD sample collection windows are defined in Table 10.

[aa] BMP urine sample collection to occur prior to dosing with lomustine on Cycle 1-Day 1 and prior to in-clinic USL311 dosing on Day 1 of every cycle thereafter.

[bb] Confirmation of archival tumor tissue availability is required prior to enrollment.

[cc] The EOT visit will occur within ≤ 14 days of the decision to discontinue study treatment; the Follow-up visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up visit; however, the Follow-up visit should occur as close as possible to 28 days after the last USL311 dose. Any TEAEs ongoing at the EOT visit should be followed to resolution or until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[dd] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival follow-up information will be collected via telephone calls and/or clinic visits.

Part 3 Schedules of Events (GBM Dose-Expansion Single Agent USL311)

Table 4: GBM Dose-Expansion Single Agent Oral USL311 – Pre-surgical Dosing Cohort

Procedures/ Assessments[a]	Screening[b]	Pre-Surgical Dosing[c,d,e]			Surgery[d,f]	Cycle 1 Post-Surgery[e,f]			Cycle 2+ Post-Surgery[e]		EOT[dd]	Follow-Up[dd]	Long-term Follow-up[ee]
	Days -28 to -1	Day 1	Days 2, 3 & 5	Day 8+		Day 1	Day 2	Days 8 & 15	Day 1	Days 8 & 15			
Informed consent[g]	X												
Inclusion/exclusion criteria[b]	X	X				X							
Medical history[h]	X												
Oncologic history[h,i]	X												
Physical examination[b,j]	X	X		X		X		X	X	X	X	X	
Neurological examination[b,k]	X	X		X		X		X	X	X	X	X	
Vital signs[l]	X	X		X		X		X	X	X	X	X	
Karnofsky performance status	X	X				X			X Even cycles only		X	X	
Holter-extracted ECG[m]	X	X	X	X	X	X	X	X	X	If indicated	X		
Real-time bedside ECG and heart rate monitoring[n]		X	X	X		X			If clinically indicated				
Pregnancy test[o]	X	X				X			X Even cycles only		X		
Hematology[b,p]	X	X		X		X		X	X	X	X	X	
Coagulation assessments[b,q]	X	X		X		X		X	X	X	X	X	
Serum chemistry[b,r]	X	X		X		X		X	X	X	X	X	

Procedures/ Assessments[a]	Screening[b]	Pre-Surgical Dosing[c,d,e]			Surgery[d,f]	Cycle 1 Post-Surgery[e,f]			Cycle 2+ Post-Surgery[e]		EOT[dd]	Follow-Up[dd]	Long-term Follow-up[ee]
	Days -28 to -1	Day 1	Days 2, 3 & 5	Day 8+		Day 1	Day 2	Days 8 & 15	Day 1	Days 8 & 15			
Urinalysis (dipstick)[b,s]	X	X				X			X		X		
Cytochrome P450 genotyping/ Pharmacogenomics[t]						X							
Response assessment: RANO[u]	X				X (see footnote)				Cycle 3-Day 1 then every 6 weeks (±7 d) thereafter and at 6 months (±7 d)		X		At 6 months only (±7 d)
Re-resection surgery					X								
USL311 oral administration[v,w]		X→	→	→		X→	→	→	→	→			
Adverse events[x]	X→	→	→	→	→	→	→	→	→	→	→	X	
Concomitant medications[y]	X→	→	→	→	→	→	→	→	→	→	→	X	
PK sample collection[z,aa]		X	X	X Day 8 only	X	X	X	X	X				
CSF sample					X								
Archival tumor tissue sample[bb]	X												
Resected tumor tissue sample					X								
EORTC QLQ-C30/BN20[cc]	X				X (see footnote)				Every 6 weeks (±7 d) and at 6 months (±7 d)		X		At 6 months only (±7 d)
Survival Status													X

Footnotes:

[a] All procedures and assessments will be performed before the administration of study drug, except as indicated below. When multiple assessments are planned at the same time point, ECG assessment (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point) will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal (scheduled) time point.

[b] The following Screening safety assessments are required to be repeated at Pre-surgical Dosing-Day 1 (baseline) if Screening assessments were performed > 3 days before Pre-surgical Dosing-Day 1 (1st dose of USL311): inclusion/exclusion criteria, physical exam, neurological exam, UA, hematology, coagulation and serum chemistry). If collected ≤ 3 days prior to Pre-surgical Dosing-Day 1, these assessments do not need to be repeated.

[c] The assessments within the Pre-surgical Dosing period only apply to subjects randomized to receive pre-surgical treatment with USL311. Subjects randomized to receive no USL311 treatment prior to surgery will not participate in any of the assessments within the Pre-surgical Dosing period and will not visit the clinic for these visits.

[d] For subjects randomized to pre-surgical treatment with USL311, surgical re-resection may occur at any time from Day 2 onwards; USL311 will be administered daily until surgery. Assessments within the Pre-surgical Dosing period that are pending at the time of surgery will no longer be required and will not be performed post-surgery. Subjects will continue with assessments as defined per Post-surgical Cycle 1.

[e] During the Pre-surgical Dosing period, the Day 1 and Day 2 visits will occur on consecutive days. The windows for Day 3, Day 5, and Day 8 visits (PK samples) are described in Table 11 All remaining Pre-surgical Dosing visits are dependent on the length of treatment with pre-surgical USL311 and will occur weekly within ± 3 days of the scheduled visit. Cycle 1-Day 1 and Day 2 Post-surgery visits will occur on consecutive days. All remaining visits (up to but not including the EOT visit), will occur within ± 3 days of scheduled visit.

[f] For subjects randomized to receive pre-surgical treatment with USL311, daily dosing will continue until surgery; the dose will be held on the day of surgery. Cycle 1-Day 1 Post-surgery will be initiated within ≤ 28 days of surgery.

[g] The IRB/IEC-approved ICF must be signed before any study-specific procedures or exams are performed.

[h] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until first dose of study drug will be recorded as an AE.

[i] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[j] Physical exams at Screening, Day 1 of each cycle post-surgery, and Follow-up are complete assessments. Other physical exams, including Day 1 of Pre-surgical Dosing, may be abbreviated, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit, height at Screening only.

[k] Neurological exams at Screening, Day 1 of each cycle post-surgery, and Follow-up are complete assessments. At Pre-surgical Dosing Day 1, complete neurological exams will be performed pre-dose and > 4 hours after dosing, prior to clinic discharge. At Pre-surgical Dosing-Day 8, Day 15, at each subsequent weekly Pre-Surgical visit, and at Post-surgery Cycle 1-Day 8 and Day 15, neurological exams will be performed prior to in-clinic dosing and may be abbreviated. Other scheduled neurological exams will be performed prior to in-clinic dosing only, and may be abbreviated exams, at the Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms.

[l] Vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to in-clinic USL311 dosing, and prior to clinic discharge. If PK collection is prior to vitals, wait ≥ 5 minutes before obtaining vitals. Subjects should be in a semi-recumbent position for ≥ 5 mins prior to collection. Collect additional vital signs if clinically indicated.

[m] Holter-extracted 12-lead ECGs will be performed at Screening, during the Pre-surgical Dosing period on Day 1 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4 and 6 hours post-dose, on Day 2 at 24 hours post the Pre-surgical Dosing-Day 1 dose (prior to Day 2 dose), on Day 3 (60 hours post-Day 1 dose), Day 5 (108 hours post-Day 1 dose) and Day 8 pre-dose. ECGs will also be extracted on the Day of Surgery (prior to surgery), and on Cycle 1-Day 1 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 7 hours post-dose, on Cycle 1-Day 2 at 24 hours post the Cycle 1-Day 1 dose (prior to Day 2 dose), and pre-dose on Cycle 1-Day 8, Day 15, on Cycle 2-Day 1 and at the EOT visit. The holter device will be placed at least 30 minutes prior to dosing and the subject will be placed in a resting, semi-recumbent position and will remain in a semi-recumbent position for at least 4 hours post-dose. No other assessments will be performed during each ECG collection period (i.e., from -15 to -5 minutes prior to the nominal time point). Frequency of ECGs may be increased and/or included at subsequent cycles if clinically indicated. All other electrical devices need to be removed from the bed/chair including cell phones.

[n] Real-time bedside ECGs and heart rate monitoring will be performed on provided equipment during Pre-surgical Dosing visits and on Cycle 1-Day 1 Post-surgery visit after in-clinic dosing. Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-tine bedside monitoring will be performed at subsequent cycles as clinically indicated.

[o] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed prior to dosing on Day 1 of the Pre-surgical Dosing period, prior to in-clinic dosing on Cycle 1-Day 1 Post-surgery, prior to dosing on Day 1 of every even numbered cycle (i.e., Cycle 2-Day 1; Cycle 4-Day 1; Cycle 6-Day 1; etc.), and at the EOT visit. If treatment with study drug is stopped for ≥ 14 days, subject should have a negative pregnancy test prior to restarting study drug.

[p] Hematology performed weekly by local laboratory: RBC count, Hgb, Hct, MCV, platelets, WBC count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), ALC, and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring, as clinically appropriate. Hematology sample can be drawn ≤ 24 hours prior to visit.

[q] Coagulation assessments performed weekly by local laboratory: PT, aPTT/PTT, and INR. Coagulation sample can be drawn ≤ 24 hours prior to visit.

[r] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, ALP, LDH, AST, ALT, glucose, bicarbonate, BUN, uric acid, CPK, and GGT. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below LLN must be corrected to within the normal range, or above the ULN if deemed not clinically significant by the Investigator, prior to dosing. The serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[s] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up on clinically significant urine dipstick findings, which include a positive test for protein, blood, nitrite and/or leukocyte esterase.

[t] A blood sample for cytochrome P450 genotyping will be collected on Cycle 1-Day 1. An optional blood sample for future pharmacogenomics analyses may also be collected on Cycle 1-Day 1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[u] Tumor assessments to be peformed at Screening, between Surgery and Cycle 1-Day 1 Post-surgery, at Cycle 3-Day 1 Post-surgery, then every 6 weeks (± 7 days) thereafter, at 6 months (± 7 days), and at the EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using same techniques. RANO criteria will be used to establish post-surgical baseline status (i.e., radiographic assessment occurring between Surgery and Day 1 of Cycle 1 Post-surgery) and to determine response. A confirmed objective response is defined as a response that persists on repeat imaging for two assessments with a time period of at least 4 weeks between the two assessments. In the event of a confirmed response, the timing of subsequent tumor assessment will be reset at the 6-week (± 7 days) interval from confirmatory scan.

[v] Study drug will be held on the morning of clinic visits and will be administered in-clinic under fasted conditions for 2 hours before and 2 hours after the dose on Pre-surgical Dosing-Day 1, Day 2, Day 3, Day 5 and Day 8 and on Cycle 1-Days 1, 2, 8 and 15 and on Cycle 2-Day 1, after pre-dose assessments have been completed.

[w] Only subjects randomized to receive USL311 prior to surgery will receive USL311 treatment during the Pre-surgical Dosing period. Subjects randomized to no USL311 treatment prior to surgery will receive their first dose of USL311 on Cycle 1-Day 1 Post-surgery.

[x] The AE/SAE collection period is from ICF signing through the Follow-up visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up visit should be followed to resolution or until the Investigator considers them chronic or stable or judges them to be no longer clinically significant.

[y] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose of USL311 until the Follow-up visit. For primary brain tumor subjects, corticosteroid use, including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[z] Blood samples (approximately 4 mL) for PK analyses (USL311) will be collected during Pre-surgical Dosing on Day 1, Day 2, Day 3, Day 5, and Day 8, on the Day of Surgery, and Post-surgery on Cycle 1-Day 1, Day 2, Day 8, and Day 15, and on Cycle 2-Day 1. Collection schedule and acceptable PK sample collection windows are as defined in Table 11 and Table 12.

[aa] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[bb] Confirmation of archival tumor tissue availability is required prior to enrollment.

[cc] The health-related QOL questionnaire will be completed at Screening, between Surgery and Post-surgery Cycle 1-Day 1, at the end of Post-surgery Cycle 2, every 6 weeks (± 7 days), at 6 months (± 7 days), and at the EOT visit. Note that these assessments should coincide with the schedule of tumor assessments, but the questionnaire should be administered prior to completing any other assessments for that visit.

[dd] The EOT visit will occur within ≤ 14 days of the decision to discontinue study treatment; the Follow-up visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up visit; however, the Follow-up visit should occur as close as possible to 28 days after the last USL311 dose . Any TEAEs ongoing at EOT visit should be followed to resolution or until the Investigator considers them chronic or stable or judges them to be no longer clinically significant.

[ee] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival information will be collected via telephone calls and/or clinic visits. If a subject discontinues treatment for non-progression reasons and has not initiated any new oncology treatment, a 6-month visit for response assessment using RANO criteria and for collecting the EORTC QLQ-C30/BN20 health-related QOL questionnaire will occur.

Table 5: GBM Dose-Expansion Single Agent USL311 – No Pre-surgical Dosing Cohort

Procedures/ Assessments[a]	Screening[b]	Surgery[d]	Cycle 1 Post-Surgery[c,d]			Cycle 2+ Post-Surgery[c]		EOT[aa]	Follow-up[aa]	Long-term Follow-up[bb]
	Days -28 to -1		Day 1	Day 2	Days 8 & 15	Day 1	Days 8 & 15			
Informed consent[e]	X									
Inclusion/exclusion criteria[b]	X	X	X							
Medical history[f]	X									
Oncologic history[f,g]	X									
Physical examination[b,h]	X	X	X		X	X	X	X	X	
Neurological examination[b,i]	X	X	X		X	X	X	X	X	
Vital signs[j]	X	X	X		X	X	X	X	X	
Karnofsky performance status	X	X	X			X Even cycles only		X	X	
Holter-extracted ECG[k]	X	X	X	X	X	X	If clinically indicated	X		
Real-time bedside ECG[l]			X			If clinically indicated				
Pregnancy test[m]	X	X	X			X Even cycles only		X		
Hematology[b,n]	X	X	X		X	X	X	X	X	
Coagulation assessments[b,o]	X	X	X		X	X	X	X	X	
Serum chemistry[b,p]	X	X	X		X	X	X	X	X	
Urinalysis (dipstick)[b,q]	X	X	X			X		X		
Cytochrome P450 genotyping/ Pharmacogenomics[r]			X							

Procedures/ Assessments[a]	Screening[b]	Surgery[d]	Cycle 1 Post-Surgery[c,d]			Cycle 2+ Post-Surgery[c]		EOT[aa]	Follow-up[aa]	Long-term Follow-up[bb]
	Days -28 to -1		Day 1	Day 2	Days 8 & 15	Day 1	Days 8 & 15			
Response assessment: RANO[s]	X	X (see footnote)				Cycle 3-Day 1, then every 6 weeks (± 7 d) thereafter and at 6 months (± 7 d)		X		At 6 months only (±7 d)
Re-resection surgery		X								
USL311 oral administration[t]			X→	→	→	→	→			
Adverse events[u]	X→	→	→	→	→	→	→	→	X	
Concomitant medications[v]	X→	→	→	→	→	→	→	→	X	
PK sample collection[w,x]		X	X	X	X	X				
CSF sample		X								
Archival tumor tissue sample[y]	X									
Resected tumor tissue sample		X								
EORTC QLQ-C30/BN20[z]	X	X (see footnote)				Every 6 weeks (± 7 d) and at 6 months (± 7 d)		X		At 6 months only (±7 d)
Survival status										X

Footnotes:

[a] All procedures and assessments will be performed before the administration of study drug, except as indicated below. When multiple assessments are planned at the same time point, ECG assessment (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point) will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal (scheduled) time point.

[b] The following Screening safety assessments are required to be repeated at Surgery (baseline) if Screening assessments were performed > 3 days before surgical re-resection: inclusion/exclusion criteria, physical exam, neurological exam, UA, hematology, coagulation and serum chemistry. If collected ≤ 3 days prior to surgery, these assessments do not need to be repeated. If the assessments need to be repeated, the pre-surgical physical exam and neurological exam performed as part of standard-of-care and performed closest to the day of surgery may be used as baseline only if it is not feasible to perform the physical and neurological exams on the day of surgery.

[c] Post-surgery Cycle 1-Day 1 and Day 2 visits will occur on consecutive days. All remaining Post-surgical Cycle 1 visits will occur on the scheduled study day or ≤ 3 days post scheduled study day. All remaining visits (Cycle 2+ up to but not including the EOT visit), will occur within ± 3 days of scheduled visit.

[d] For subjects randomized to receive no USL311 treatment prior to surgery, surgical re-resection will occur following Screening, with the Screening Day -28 to Day -1 window calculated relative to day of Surgery. The Cycle 1-Day 1 Post-surgery visit will be initiated ≤ 28 days of surgery. These subjects will not participate in any of the assessments within the Pre-surgical Dosing period and will not visit the clinic for these visits.

[e] The IRB/IEC-approved ICF must be signed before any study-specific procedures or exams are performed.

[f] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until first dose of study drug will be recorded as an AE.

[g] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[h] Physical exams at Screening, Day 1 of each cycle Post-surgery, and at Follow-up should be complete assessments. Other physical exams may be abbreviated, at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit, height at Screening only.

[i] Neurological exams at Screening, Day 1 of each cycle Post-surgery, and Follow-up will be complete assessments. At Cycle 1-Day 1, complete neurological exams will be performed pre-dose and >4 hours after dosing, prior to clinic discharge. At Post-surgery Cycle 1-Day 8 and Day 15, neurological exams will be performed prior to in-clinic dosing and may be abbreviated. Other scheduled neurological exams will be performed prior to in-clinic dosing only, and may be abbreviated exams, at the Investigator's discretion to identify changes from baseline or evaluate changes in clinical symptoms.

[j] Vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to in-clinic dosing with USL311 and prior to clinic discharge. If PK collection is prior to vitals, wait ≥ 5 minutes before obtaining vitals. Subjects should be in a semi-recumbent position for at least 5 mins prior to collection. Perform additional vital signs if clinically indicated.

[k] Holter-extracted 12-lead ECGs will be performed at Screening, on the Day of Surgery (prior to surgery), on Cycle 1-Day 1 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 7 hours post-dose, on Cycle 1-Day 2 at 24 hours post Cycle 1-Day 1 dose (prior to the Cycle 1-Day 2 dose), and predose on Cycle 1-Day 8, Cycle 1-Day 15, and Cycle 2-Day 1, and at the EOT visit. The holter device will be placed at least 30 minutes prior to dosing and the subject will be placed in a resting, semi-recumbent position and will remain in a semi-recumbent position for at least 4 hours post-dose. No other assessments will be performed during each ECG collection period (i.e., from -15 to -5 minutes prior to the nominal time point). Frequency of ECGs may be increased and/or included at subsequent cycles if clinically indicated. All other electrical devices need to be removed from the bed/chair including cell phones.

[l] Real-time bedside ECGs and heart rate monitoring will be performed on provided equipment on Cycle 1-Day 1 Post-surgery after in-clinic dosing. Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-time bedside monitoring will be performed at subsequent cycles as clinically indicated.

[m] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed on the day of Surgery, prior to dosing on Cycle 1-Day 1 Post-surgery, prior to dosing on Day 1 of every even numbered cycle (i.e., Cycle 2-Day 1; Cycle 4-Day 1; Cycle 6-Day 1; etc.), and at EOT visit. If treatment with study drug is stopped for ≥ 14 days, subject should have a negative pregnancy test prior to restarting drug.

[n] Hematology performed weekly by local laboratory: RBC count, Hgb, Hct, MCV, platelets, WBC count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), ALC, and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring, as clinically appropriate. Hematology sample can be drawn ≤ 24 hours prior to visit.

[o] Coagulation performed weekly by local laboratory: PT, aPTT/PTT, and INR. Coagulation sample can be drawn ≤ 24 hours prior to dosing.

[p] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, ALP, LDH, AST, ALT, glucose, bicarbonate, BUN, uric acid, CPK, and GGT. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below LLN must be corrected to within the normal range, or above the ULN if deemed not clinically significant by the Investigator, prior to dosing. Serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[q] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up clinically significant urine dipstick findings, which include a positive test for protein, blood, nitrite and/or leukocyte esterase.

[r] A blood sample for cytochrome P450 genotyping will be collected on Cycle 1-Day 1. An optional blood sample for future pharmacogenomics analyses may also be collected on Cycle 1-Day 1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[s] Tumor assessments to be performed at Screening, between Surgery and Cycle 1-Day 1 Post-surgery, at Cycle 3-Day 1 Post-surgery, then every 6 weeks (± 7 days) thereafter, at 6 months (± 7 days), and at EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using the same techniques. RANO criteria will be used to determine response. A confirmed objective response is defined as a response that persists on repeat imaging for two assessments with a time period of at least 4 weeks between the two assessments. In the event of a confirmed response, the timing of subsequent tumor assessment will be reset at the 6-week (± 7 days) interval from confirmatory scan.

[t] Study drug will be held on the morning of clinic visits and will be administered in-clinic under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 1, 2, 8 and 15 and on Cycle 2-Day 1, after pre-dose assessments have been completed.

[u] The AE/SAE collection period is from the ICF signing through the Follow-up visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up visit should be followed to resolution, until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[v] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose of USL311 until the Follow-up visit. For primary brain tumor and GBM subjects, corticosteroid use, including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[w] Blood samples (approximately 4 mL) for PK analyses (USL311) will be collected on the Day of Surgery and Post-surgery on Cycle 1-Day 1, Day 2, Day 8, and Day 15 and on Cycle 2-Day 1. Collection schedule and acceptable PK sample collection windows are as defined in Table 12.

[x] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[y] Confirmation of archival tumor tissue availability is required prior to enrollment.

[z] The health-related QOL questionnaire will be completed at Screening, between Surgery and Post-surgery Cycle 1-Day 1, at the end of Post-surgery Cycle 2, every 6 weeks (± 7 days), at 6 months (± 7 days), and at the EOT Visit. Note that these assessments should coincide with the schedule of tumor assessments but the questionnaire should be administered prior to completing any other assessments for that visit.

[aa] The EOT Visit will occur ≤ 14 days of the decision to discontinue study treatment; the Follow-up visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up visit; however, the Follow-up visit should occur as close as possible to 28 days after the last USL311 dose. Any TEAEs ongoing at EOT visit should be followed to resolution or until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[bb] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival information will be collected via telephone calls and/or clinic visits. If a subject discontinues treatment for non-progression reasons and has not initiated any new oncology treatment, a 6-month visit for response assessment using RANO criteria and for collecting the EORTC QLQ-C30/BN20 health-related QOL questionnaire will occur.

Part 4 Schedules of Events (GBM Dose-Expansion USL311 + Lomustine)

Table 6: GBM Dose-Expansion USL311 + Lomustine – Pre-surgical Dosing Cohort

Procedures/ Assessments[a]	Screening[b]	Pre-surgical Dosing (Single-Agent USL311 only)[c,d,e]			Surgery[d,f]	Cycle 1 Post-Surgery (USL311 + Lomustine)[e,f]				Cycle 2 + Post-Surgery[d]			EOT[ee]	Follow-up[ee]	Long-term Follow-up[ff]
	Days -28 to -1	Day 1	Days 2, 3 & 5	Day 8+		Day 1	Day 2	Days 8 & 15	Days 22, 29 & 36	Day 1	Day 2	Days 8, 15, 22, 29 & 36			
Informed consent[g]	X														
Inclusion/exclusion criteria[b]	X	X				X									
Medical history[h]	X														
Oncologic history[h,i]	X														
Physical examination[b,j]	X	X		X		X		X	X	X		X	X	X	
Neurological examination[b,k]	X	X		X		X		X	X	X		X	X	X	
Vital signs[l]	X	X		X		X		X	X	X		X	X	X	
Karnofsky performance status	X	X				X				X			X	X	
Holter-extracted ECG[m]	X	X	X	X Day 8 only	X	X	X	X		X	If indicated		X		
Real-time bedside ECG[n]		X		X		X				If indicated					
Pregnancy test[o]	X	X				X				X			X		
Hematology[b,p]	X	X		X		X		X	X	X		X	X	X	
Coagulation assessments[b,q]	X	X		X		X		X	X	X		X	X	X	
Serum chemistry[b,r]	X	X		X		X		X	X	X		X	X	X	
Urinalysis (dipstick)[b,s]	X	X				X				X			X		
Cytochrome P450 genotyping/ Pharmacogenomics[t]						X									

Procedures/ Assessments[a]	Screening[b]	Pre-surgical Dosing (Single-Agent USL311 only)[c,d,e]			Surgery[d,f]	Cycle 1 Post-Surgery (USL311 + Lomustine)[e,f]				Cycle 2 + Post-Surgery[d]			EOT[ee]	Follow-up[ee]	Long-term Follow-up[ff]
	Days -28 to -1	Day 1	Days 2, 3 & 5	Day 8+		Day 1	Day 2	Days 8 & 15	Days 22, 29 & 36	Day 1	Day 2	Days 8, 15, 22, 29 & 36			
Response assessment: RANO[u]	X				X (see footnote)					Cycle 2-Day 1 then every 6 weeks (±7 d) thereafter and at 6 months (±7 d)			X		At 6 months only (±7 d)
Re-resection surgery					X										
USL311 oral administration[v,w]		X→	→	→		X→	→	→	→	→	→	→			
Lomustine administration[x]							X				X				
Adverse events[y]	X→	→	→	→	→	→	→	→	→	→	→	→	→	X	
Concomitant medications[z]	X→	→	→	→	→	→	→	→	→	→	→	→	→	X	
USL311 PK blood sample collection[aa,bb]		X	X	X Day 8 only	X	X	X	X		X					
Lomustine PK blood sample collection[aa,bb]							X								
CSF sample					X										
Archival tumor tissue sample[cc]	X														
Resected tumor tissue sample					X										
EORTC QLQ-C30/BN20[dd]	X				X (see footnote)					Every 6 weeks (±7 d) and at 6 months (±7 d)			X		At 6 months only (±7 d)
Survival status															X

Footnotes:

[a] All procedures and examinations will be performed before the administration of study drug, except as indicated below. When multiple assessments are planned at the same time point, ECG assessment (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point) will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal time point.

[b] The following Screening safety assessments are required to be repeated at Pre-surgical Dosing-Day 1 (baseline) if Screening assessments were performed > 3 days before Pre-surgical Dosing-Day 1 (1st dose of USL311): inclusion/exclusion criteria, physical exam, neurological exam, UA, hematology, coagulation and serum chemistry). If collected ≤ 3 days prior to Pre-surgical Dosing-Day 1, these assessments do not need to be repeated.

[c] The assessments within the Pre-surgical Dosing period only apply to subjects randomized to receive pre-surgical treatment with USL311. Subjects randomized to receive no USL311 treatment prior to surgery will not participate in any of the assessments within the Pre-surgical Dosing period and will not visit the clinic for these visits.

[d] For subjects randomized to pre-surgical treatment with USL311, surgical re-resection may occur at any time from Day 2 onwards; USL311 will be administered daily until surgery. Assessments within the Pre-surgical Dosing period that are pending at the time of surgery will no longer be required and will not be performed post-surgery. Subjects will continue with assessments as defined per Post-surgical Cycle 1.

[e] During the Pre-Surgical Dosing period, the Day 1 and Day 2 visits will occur on consecutive days. The windows for Day 3, Day 5, and Day 8 visits (PK samples) are described in Table 11. All remaining Pre-surgical Dosing visits are dependent on the length of treatment with pre-surgical USL311 and will occur weekly after Day 8 (e.g., Day 15, 22, etc.) a minimum of 7 days and a maximum of 14 days from the previous visit. Cycle 1-Day 1 and Day 2 Post-surgery visits will occur on consecutive days All remaining visits (up to but not including the EOT visit), will occur within ± 3 days of scheduled visit.

[f] For subjects randomized to receive pre-surgical treatment with USL311, daily dosing will continue until surgery; the dose will be held on the day of surgery. Cycle 1-Day 1 Post-surgery will be initiated within ≤ 28 days of surgery.

[g] The IRB/IEC-approved ICF must be signed before any study-specific procedures or exams are performed.

[h] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until the first dose of study drug will be recorded as an AE .

[i] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[j] Physical exams at Screening, Day 1 of each cycle post-surgery, and at Follow-up are complete assessments. Other physical exams, including Day 1 of Pre-surgical Dosing, may be abbreviated at Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit, height at Screening only.

[k] Neurological exams at Screening, Day 1 of each cycle post-surgery, and Follow-up are complete assessments. At Pre-surgical Dosing Day 1, complete neurological exam will be performed pre-dose and > 4 hours after dosing, prior to clinic discharge. At Pre-surgical Dosing-Day 8, Day 15 and at each subsequent weekly Pre-Surgical visit, and at Post-surgery Cycle 1-Day 8, Day 15, Day 22, Day 29, and Day 36, neurological exams will be performed prior to in-clinic dosing and may be abbreviated. Other scheduled neurological exams will be performed prior to in-clinic dosing only, and may be abbreviated exams at the Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms.

[l] Vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to in-clinic USL311 dosing, and prior to clinic discharge. If PK collection is prior to vitals, wait ≥ 5 minutes before obtaining vitals. Subjects should be in a semi-recumbent position for ≥ 5 mins prior to collection. Collect additional vital signs if clinically indicated.

[m] Holter-extracted 12-lead ECGs will be performed at Screening, during the Pre-surgical Dosing period on Day 1 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4 and 6 hours post-dose, on Day 2 at 24 hours post the Pre-surgical Dosing-Day 1 dose (prior to Day 2 dose), on Day 3 (60 hours post-Day 1 dose), Day 5 (108 hours post-Day 1 dose) and Day 8 pre-dose. ECGs will also be extracted on the day of Surgery (prior to surgery), on Cycle 1-Day 1 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 7 hours post-dose, on Cycle 1-Day 2 at 24 hours post the Cycle 1-Day 1 dose (prior to the Cycle 1-Day 2 dose), and pre-dose on Cycle 1-Day 8, Cycle 1-Day 15, Cycle 2-Day 1 and at the EOT visit. The holter device will be placed at least 30 minutes prior to dosing and the subject will be placed in a resting, semi-recumbent position and will remain in a semi-recumbent position for at least 4 hours post-dose. No other assessments will be performed during each ECG collection period (i.e., from -15 to -5 minutes prior to the nominal time point). Frequency of ECGs may be increased and/or included at subsequent cycles if clinically indicated. All other electrical devices need to be removed from the bed/chair including cell phones.

[n] Real-time bedside ECGs and heart rate monitoring will be performed on provided equipment during the Pre-surgical Dosing visits and on Cycle 1-Day 1 Post-surgery visit after in-clinic dosing. Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-tine bedside monitoring will be performed at subsequent cycles as clinically indicated.

[o] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed prior to dosing on Day 1 of the Pre-surgical Dosing period, prior to in-clinic USL311 dosing on Day 1 of every cycle thereafter, and at the EOT visit. If treatment with study drug is stopped for ≥ 14 days, subject should have a negative pregnancy test prior to restarting drug.

[p] Hematology performed weekly by local laboratory: RBC count, Hgb, Hct, MCV, platelets, WBC count, WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), ALC, and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring, as clinically appropriate. Hematology sample can be drawn ≤ 24 hours prior to visit.

[q] Coagulation performed weekly by local laboratory: PT, aPTT/PTT and INR. Coagulation sample can be drawn ≤ 24 hours prior to visit.

[r] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, ALP, LDH, AST, ALT, glucose, bicarbonate, BUN, uric acid, CPK, and GGT. Baseline sodium, potassium, calcium (corrected for albumin) and magnesium levels below LLT must be corrected to within the normal range, or above the ULN if deemed not clinically significant by the Investigator, prior to dosing. Serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[s] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up on clinically significant urine dipstick findings, which include a positive test for protein, blood, nitrite and/or leukocyte esterase.

[t] A blood sample for cytochrome P450 genotyping will be collected on Cycle 1-Day 1. An optional blood sample for future pharmacogenomics analyses may also be collected on Cycle 1-Day 1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[u] Tumor assessments to be performed at Screening, between Surgery and Cycle 1-Day 1 Post-surgery, at Cycle 2-Day 1 Post-surgery, then every 6 weeks (± 7 days) thereafter, at 6 months (± 7 days), and at the EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using same techniques. RANO criteria will be used to establish post-surgical baseline status (i.e., radiographic assessment occurring between Surgery and Cycle 1-Day 1 Post-surgery) and to determine response. A confirmed objective response is defined as a response that persists on repeat imaging for two assessments with a time period of at least 4 weeks between the two assessments. In the event of a confirmed response, the timing of subsequent tumor assessment will be reset at the 6-week (± 7 days) interval from confirmatory scan.

[v] Study drug will be held on the morning of clinic visits and will be administered in-clinic under fasted conditions for 2 hours before and 2 hours after the dose on Pre-surgical Dosing-Day 1, Day 2, Day 3, Day 5 and Day 8 and on Cycle 1-Days 1, 2, 8 and 15 and on Cycle 2-Day 1, after pre-dose assessments have been completed.

[w] Only subjects randomized to receive USL311 prior to surgery will receive USL311 treatment during the Pre-surgical period. Subjects randomized to no USL311 treatment prior to surgery will receive their first dose of USL311 on Cycle 1-Day 1 Post-surgery.

[x] Lomustine will be given every 6 weeks and will be administered on Day 2 of each cycle Post-surgery.

[y] The AE/SAE collection period is from ICF signing through the Follow-up visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up visit should be followed to resolution or until the Investigator considers them chronic or stable or judges them to be no longer clinically significant.

[z] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose of USL311 until the Follow-up visit. For primary brain tumor subjects, corticosteroid use, including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[aa] Blood samples (approximately 4 ml) for PK analyses will be collected during Pre-surgical Dosing on Day 1, Day 2, Day 3, Day 5, and Day 8, on the Day of Surgery,and Post-surgery on Cycle 1-Day 1, Day 2, Day 8, and Day 15, and on Cycle 2-Day 1. Collection schedule and acceptable PK sample collection windows are as defined in Table 11 and Table 12.

[bb] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[cc] Confirmation of archival tumor tissue availability is required prior to enrollment.

[dd] The health-related QOL questionnaire will be completed at Screening, between Surgery and Post-Surgery Cycle 1-Day 1, at the end of Post-Surgery Cycle 2, every 6 weeks (± 7 days), at 6 months (± 7 days), and at the EOT visit. Note that these assessments should coincide with the schedule of tumor assessments but the questionnaire should be administered prior to completing any other assessments for that visit.

[ee] The EOT visit will occur within ≤ 14 days of the decision to discontinue study treatment; the Follow-up visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up visit; however, the Follow-up visit should occur as close as possible to 28 days after the last USL311 dose. Any TEAEs ongoing at EOT visit should be followed to resolution or until the Investigator considers them chronic or stable, or judges them to be no longer clinically significant.

[ff] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival information will be collected via telephone calls and/or clinic visits. If a subject discontinues treatment for non-progression reasons and has not initiated any new oncology treatment, a 6-month visit for response assessment using RANO criteria and for collecting the EORTC QLQ-C30/BN20 health-related QOL questionnaire will occur.

Table 7: GBM Dose-Expansion USL311 + Lomustine – No Pre-surgical Dosing Cohort

Procedures/ Assessments[a]	Screening[b]	Surgery[d]	Cycle 1 Post-surgery (USL311 + Lomustine)[c,d]				Cycle 2 + Post-surgery[c]				End of Tx[bb]	Follow-up[bb]	Long-term Follow-up[cc]
	Days -28 to -1		Day 1	Day 2	Days 8 & 15	Days 22, 29 & 36	Day 1	Day 2	Days 8, 15, 22 & 29	Day 36			
Informed consent[e]	X												
Inclusion/exclusion criteria[b]	X	X	X										
Medical history[f]	X												
Oncologic history[f,g]	X												
Physical examination[b,h]	X	X	X		X	X	X		X	X	X	X	
Neurological examination[b,i]	X	X	X		X	X	X		X	X	X	X	
Vital signs[j]	X	X	X		X	X	X		X	X	X	X	
Karnofsky performance status	X	X	X				X				X	X	
Holter-extracted ECG[k]	X	X	X	X	X		X	If indicated			X		
Real-time bedside ECG[l]			X				If indicated						
Pregnancy test[m]	X	X	X				X				X		
Hematology[b,n]	X	X	X		X	X	X		X	X	X	X	
Coagulation assessments[b,o]	X	X	X		X	X	X		X	X	X	X	
Serum chemistry[b,p]	X	X	X		X	X	X		X	X	X	X	
Urinalysis (dipstick)[b,q]	X	X	X				X				X		
Cytochrome P450 genotyping/ Pharmacogenomics[r]			X										
Response assessment: RANO[s]	X	X (see footnote)					Cycle 2-Day 1, then every 6 weeks (± 7 d) thereafter and at 6 months (± 7 d)				X		At 6 months only (± 7 d)
Re-resection surgery		X					→	→	→	→			

Procedures/ Assessments[a]	Screening[b]	Surgery[d]	Cycle 1 Post-surgery (USL311 + Lomustine)[c,d]				Cycle 2 + Post-surgery[c]				End of Tx[bb]	Follow-up[bb]	Long-term Follow-up[cc]
	Days -28 to -1		Day 1	Day 2	Days 8 & 15	Days 22, 29 & 36	Day 1	Day 2	Days 8, 15, 22 & 29	Day 36			
USL311 oral administration[t]			X→	→	→	→	→	→	→	→			
Lomustine administration[u]				X				X					
Adverse events[v]	X→	→	→	→	→	→	→	→	→	→	→	X	
Concomitant medications[w]	X→	→	→	→	→	→	→	→	→	→	→	X	
USL311 PK blood sample collection[x,y]		X	X	X	X		X						
Lomustine PK blood sample collection[x,y]				X									
CSF sample		X											
Archival tumor tissue sample[z]	X												
Resected tumor tissue sample		X											
EORTC QLQ-C30/BN20[aa]	X	X (see footnote)					Every 6 weeks (±7 d) and at 6 months (±7 d)				X		At 6 months only (±7 d)
Survival status													X

Footnotes:

[a] All procedures and examinations will be performed before the administration of study drug, except as indicated below. When multiple assessments are planned at the same time point, ECG assessment (including semi-recumbent positioning which is to occur at least 20 minutes prior to the nominal time point), will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal time point.

[b] The following Screening safety assessments are required to be repeated at Surgery (baseline) if Screening assessments were performed > 3 days before surgical re-resection: inclusion/exclusion criteria, ECG, vital signs, physical exam, neurological exam, UA, hematology, coagulation, and serum chemistry. If collected ≤ 3 days prior to surgery, these assessments do not need to be repeated. If the assessments need to be repeated, the pre-surgical physical exam and neurological exam performed as part of standard-of-care and performed closest to the day of surgery may be used as baseline only if it is not feasible to perform the physical and neurological exams on the day of surgery.

[c] Post-surgical Cycle 1-Day 1 and Day 2 visits will occur on consecutive days. All remaining Post-surgical Cycle 1 visits should occur on the scheduled study day or ≤ days post scheduled study day. All remaining visits (Cycle 2+ up to but not including the EOT visit), may occur within ± 3 days of scheduled visit.

[d] For subjects randomized to receive no USL311 treatment prior to surgery, Surgery (re-resection) will occur following Screening, with the Screening Day -28 to Day -1 window calculated relative to day of Surgery. The Cycle 1-Day 1 Post-surgery visit will be initiated ≤ 28 days of surgery. These subjects will not participate in any of the assessments within the Pre-surgical Dosing period and will not visit the clinic for these visits.

[e] The IRB/IEC-approved ICF must be signed before any study-specific procedures or exams are performed.

[f] New or clinically significant changes in subject's medical and/or oncologic history from ICF signing until first dose of study drug will be recorded as an AE .

[g] Oncologic history includes disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For each systemic therapy, the date of last therapy and best response to therapy should be documented, if possible. Any residual toxicity related to prior treatments should be documented.

[h] Physical exams at Screening, Day 1 of each cycle Post-surgery, and at Follow-up should be complete assessments. Other physical exams may be abbreviated, at the Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms. Weight, BMI, and BSA to be reported at each visit, height at Screening only.

[i] Neurological exams at Screening, Day 1 of each cycle Post-surgery, and Follow-up will be complete assessments. During Cycle 1-Day 1, a complete neurological exam will be performed pre-doseand >4 hours after dosing, prior to clinic discharge. At Post-surgery Cycle 1-Day 8, Day 15, Day 22, Day 29 and Day 36, neurological exams will be performed prior to in-clinic dosing and may be abbreviated. Other scheduled neurological exams will be performed prior to in-clinic dosing only, and may be abbreviated exams, at the Investigator's discretion, to identify changes from baseline or evaluate changes in clinical symptoms.

[j] Vital signs (pulse, systolic and diastolic blood pressure, temperature, and respiration rate) will be collected prior to in-clinic dosing with USL311, and prior to clinic discharge. If PK collection is prior to vitals, wait ≥ 5 minutes before obtaining vitals. Subjects should be in a semi-recumbent position for at least 5 mins prior to collectionPerform additional vital signs assessments if clinically indicated.

[k] Holter-extracted 12-lead ECGs will be performed at Screening, on the day of Surgery (prior to surgery), on Cycle 1-Day 1 pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 and 7 hours post-dose, on Cycle 1-Day 2, 24 hours post the Cycle 1-Day 1 dose (prior to the Cycle 1-Day 2 dose), on Cycle 1-Day 8, Cycle 1-Day 15, Cycle 2-Day 1 and at the EOT visit. The holter device will be placed at least 30 minutes prior to dosing and the subject will be placed in a resting, semi-recumbent position and will remain in a semi-recumbent position for at least 4 hours post-dose. No other assessments will be performed during each ECG collection period (i.e., from -15 to -5 minutes prior to the nominal time point). Frequency of ECGs may be increased and/or included at subsequent cycles if clinically indicated. All other electrical devices need to be removed from the bed/chair including cell phones.

[l] Real-time bedside ECGs and heart rate monitoring will be performed on the provided equipment during the Cycle 1-Day 1 Post-surgery visit after in-clinic dosing. Printouts may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Real-tine bedside monitoring will be performed at subsequent cycles as clinically indicated.

[m] For women of childbearing potential, serum pregnancy test is required at Screening. Serum or urine pregnancy test to be performed on the day of Surgery, prior to dosing on Day 1 of every cycle thereafter, and at the EOT visit. If treatment with study drug is stopped for ≥ 14 days, subject should have a negative pregnancy test prior to restarting drug.

[n] Hematology performed weekly by local laboratory: RBC count, Hgb, Hct, MCV, platelets, WBC count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), ALC, and ANC. If weekly hematology demonstrates CTCAE grade 3/4 cytopenias, increase to daily monitoring as clinically appropriate. Hematology sample can be drawn ≤ 24 hours prior to visit.

[o] Coagulation performed weekly by local laboratory: PT, aPTT/PTT, INR. Coagulation sample can be drawn ≤ 24 hours prior to dosing.

[p] Serum chemistry performed weekly by local laboratory: sodium, potassium, calcium, chloride, phosphorus or phosphate, magnesium, serum creatinine, total bilirubin, total protein, albumin, ALP, LDH, AST, ALT, glucose, bicarbonate, BUN, uric acid, CPK, and GGT. Baseline sodium, potassium, calcium (corrected for albumin) and magnesium levels below LLN must be corrected to within the normal range, or above the ULN if determined not clinically significant by the Investigator, prior to dosing. Serum chemistry sample can be drawn ≤ 24 hours prior to dosing.

[q] Urine dipstick must include pH, sp gravity, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase. Microscopy is required only to follow-up clinically significant urine dipstick findings, which include a positive test for protein, blood, nitrite and/or leukocyte esterase.

[r] A blood sample for cytochrome P450 genotyping will be collected on Cycle 1-Day 1. An optional blood sample for future pharmacogenomics analyses may also be collected on Cycle 1-Day 1 (or at a later visit if determined appropriate per the clinical site) after appropriate separate consent is obtained.

[s] Tumor assessments to be performed at Screening, between Surgery and Cycle 1-Day 1 Post-surgery, at Cycle 2-Day 1 Post-surgery, then every 6 weeks (± 7 days) thereafter, at 6 months (± 7 days), and at the EOT visit. Baseline and subsequent radiographs/scans to assess response should be performed using same techniques. RANO criteria will be used to establish post-surgical baseline status (i.e., between Surgery and Cycle 1-Day 1 Post-surgery) and to determine response. A confirmed objective response is defined as a response that persists on repeat imaging for two assessments with a time period of at least 4 weeks between the two assessments. In the event of a confirmed response, the timing of subsequent tumor assessment will be reset at the 6-week (± 7 days) interval from confirmatory scan.

[t] Study drug will be held on the morning of clinic visits and will be administered in-clinic under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 1, 2, 8 and 15 and on Cycle 2-Day 1, after pre-dose assessments have been completed.

[u] Lomustine will be given every 6 weeks and will be administered on Day 2 of each cycle Post-surgery.

[v] The AE/SAE collection period is from ICF signing through the Follow-up visit. AEs/SAEs must be reported as described in Section 9.3 of the protocol. Any AEs/SAEs ongoing at the Follow-up visit should be followed to resolution or until the Investigator considers them chronic or stable or judges them to be no longer clinically significant.

[w] All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose of USL311 until the Follow-up visit. For primary brain tumor subjects, corticosteroid use, including any changes in dose and/or frequency will be documented for RANO criteria assessment.

[x] Blood samples (approximately 4 mL) for PK analyses will be collected on the Day of Surgery, and Post-surgery on Cycle 1-Day 1, Day 2, Day 8, and Day 15, and on Cycle 2-Day 1. Collection schedule and acceptable PK sample collection windows are as defined in Table 12.

[y] In the event of a possible study drug-related SAE or DLT throughout the study, attempts will be made to collect additional PK blood samples as close to the event as possible to help characterize any possible relationships between drug exposure and the clinical event.

[z] Confirmation of archival tumor tissue availability is required prior to enrollment.

[aa] The health-related QOL questionnaire will be completed at Screening, between Surgery and Cycle 1-Day 1 Post-surgery, at the end of Cycle 2 Post-surgery, every 6 weeks (± 7 days), at 6 months (± 7 days), and at the EOT visit. Note that these assessments should coincide with the schedule of tumor assessments but the questionnaire should be administered prior to completing any other assessments for that visit. [bb] The EOT visit will occur ≤ 14 days of the decision to discontinue study treatment; the Follow-up visit will occur within ≤ 28 days of the last USL311 dose. The EOT visit may be the same as the Follow-up visit; however, the Follow-up visit should occur as close as possible to 28 days after the last USL311 dose. Any TEAEs ongoing at EOT visit should be followed to resolution or until the Investigator considers them chronic or stable or judges them to be no longer clinically significant.

[cc] Subjects will be followed for survival information quarterly as defined in Section 7.4. Survival information will be collected via telephone calls and/or clinic visits. If a subject discontinues treatment for non-progression reasons and has not initiated any new oncology treatment, a 6-month visit for response assessment using RANO criteria and for collecting the EORTC QLQ-C30/BN20 health-related QOL questionnaire will occur.

Pharmacokinetic (PK) and Pharmacodynamic (PD) Sample Times

Table 8: PK and PD Sample Times – Part 1a: IV USL311 Single Agent Dose Escalation

Day	Sample	USL311 PK	WBC	SDF-1	CD34+	Nominal Time[a]	Acceptable Collection Window
Cycle 1, Day 1	1	X	X	X	X	Predose	≤ 30 min prior to USL311 infusion
	2	X	X	NS	NS	0.5 hr	± 2 min
	3	X	X	NS	NS	1 hr	± 5 min
	4	X	X	X	X	2 hr	± 5 min
	5	X	X	NS	NS	3 hr	± 5 min
	6	X	X	X	X	4 hr	± 5 min
	7	X	X	NS	NS	5 hr	± 5 min
	8	X	X	X	X	6 hr	± 5 min
	9	X	X	NS	NS	7 hr	± 5 min
	10	X	X	X	X	8 hr	± 5 min
Cycle 1, Day 2	11	X	X	X	X	24 hr	± 60 min
Cycle 1, Day 3	12	X	X	NS	NS	60 hr	≥ 48 hr to < 72 hr[a,b]
Cycle 1, Day 5	13	X	X	NS	NS	108 hr	≥ 96 hr to < 120 hr[a,b]
Cycle 1, Day 8	14	X	X	NS	NS	Predose (Equivalent to Dose #1 trough)	≤ 30 min prior to USL311 infusion (Dose #2)
	15	X	NS	NS	NS	0.5 hr	± 2 min
	16	X	NS	NS	NS	1.5 hr	± 5 min
	17	X	X	NS	NS	3 hr	± 5 min
	18	X	X	NS	NS	4 hr	± 5 min
Cycle 1 Day 15	19	X	X	NS	NS	Predose (Equivalent to Dose #2 trough)	≤ 30 min prior to USL311 infusion (Dose #3)
	20	X	X	NS	NS	2 hr	± 5 min
	21	X	X	NS	NS	4 hr	± 5 min
Cycle 2, Day 1	22	X	X	NS	NS	Predose (Equivalent to Dose #3 trough)	≤ 30 min prior to USL311 infusion (Dose #4)

Table 9: PK and PD Sample Times – Part 1b: Oral USL311 Single Agent Dose Escalation

Day	Sample	USL311 PK	WBC	CD34+	Nominal Time[a]	Acceptable Collection Window
Cycle 1-Day 1	1	X	X	X	Predose	≤ 30 min prior to USL311 dose
	2	X	X	NS	0.5 hr	± 2 min
	3	X	X	NS	1 hr	± 5 min
	4	X	X	NS	1.5 hr	± 5 min
	5	X	X	X	2 hr	± 5 min
	6	X	X	NS	2.5 hr	± 5 min
	7	X	X	NS	3 hr	± 5 min
	8	X	X	X	4 hr	± 5 min
	9	X	X	X	6 hr	± 5 min
Cycle 1-Day 2	10	X	X	X	24 hr	± 60 min Prior to Day 2 dose
Cycle 1-Day 8	11	X	NS	NS	Predose	≤ 30 min prior to USL311 dose
	12	X	NS	NS	0.5 hr	± 5 min
	13	X	X	NS	2 hr	± 5 min
	14	X	X	NS	3 hr	± 5 min
	15	X	X	NS	4 hr	± 5 min
Cycle 1-Day 15	16	X	X	NS	Predose	≤ 30 min prior to USL311 dose
	17	X	X	NS	2-4 hr[c]	± 5 min
Cycle 2-Day 1	18	X	X	NS	Predose	≤ 30 min prior to USL311 dose
	19	X	X	NS	2-4 hr[c]	± 5 min

NS = No Sample Collected

[a] All nominal times are relative to the start of USL311 infusion

[b] One sample to be collected anytime within the specified time window

[b] The exact collection time will be decided after reviewing the PK data obtained on Cycle 1-Day 1

Table 10: PK and PD Sample Times – Part 2: USL311 + Lomustine Dose Escalation

Day	Sample	Lomustine PK	USL311 PK	WBC	CD34 +	Nominal Time	Acceptable collection window
Cycle 1-Day 1	1	X	NS	NS	NS	Predose	≤ 24 hr prior to lomustine administration
	2	X	NS	NS	NS	1 hr	± 5 min (nominal time is relative to lomustine dose administration)
	3	X	NS	NS	NS	2 hr	
	4	X	NS	NS	NS	3 hr	
	5	X	NS	NS	NS	4 hr	
	6	X	NS	NS	NS	5 hr	
	7	X	NS	NS	NS	8 hr	
	8	X	NS	NS	NS	12 hr	
Cycle 1-Day 8	9	NS	X	X	X	Predose	≤ 30 min prior to USL311 administration
	10	NS	X	X	NS	0.5 hr	± 5 min (nominal time is relative to USL311 administration)
	11	NS	X	X	X	1 hr	
	12	NS	X	X	NS	1.5 hr	
	13	NS	X	X	NS	2 hr	
	14	NS	X	X	NS	2.5 hr	
	15	NS	X	X	NS	3 hr	
	16	NS	X	X	X	4 hr	
	17	NS	X	X	NS	6 hr	
Cycle 1-Day 9	18	NS	X	X	X	24 hr	± 60 min
Cycle 1-Day 15	19	NS	X	X	X	Predose	≤ 30 min prior to USL311 administration
	20	NS	X	NS	NS	0.5 hr	± 5 min (nominal time is relative to USL311 administration)
	21	NS	X	X	NS	2 hr	
	22	NS	X	NS	NS	3 hr	
	23	NS	X	X	NS	4 hr	
Cycle 1-Day 22	24	NS	X	X	X	Predose	≤ 30 min prior to USL311 administration
	25	NS	X	X	NS	2-4 hr[a]	± 5 min (nominal time is relative to USL311 administration)
Cycle 2-Day 1	26	NS	X	X	NS	Predose	≤ 30 min prior to USL311 administration
	27	NS	X	NS	NS	0.5 hr	± 5 min (nominal time is relative
	28	NS	X	X	NS	1 hr	

Day	Sample	Lomustine PK	USL311 PK	WBC	CD34 +	Nominal Time	Acceptable collection window
	29	NS	X	X	NS	1.5 hr	to USL311 administration)
	30	NS	X	NS	NS	2 hr	
	31	NS	X	X	NS	2.5 hr	
	32	NS	X	X	NS	3 hr	
	33	NS	X	X	NS	4 hr	
	34	NS	X	X	NS	6 hr	
Cycle 2-Day 2	35	X	X	NS	NS	24 hr/ Predose	≤ 30 min prior to lomustine administration
	36	X	NS	NS	NS	1 hr	± 5 min (nominal time is relative to lomustine dose administration or USL311 administration where applicable)
	37	X	NS	NS	NS	2 hr	
	38	X	NS	NS	NS	3 hr	
	39	X	NS	NS	NS	4 hr	
	40	X	NS	NS	NS	5 hr	
	41	X	NS	NS	NS	8 hr	
	42	X	NS	NS	NS	12 hr	

NS = No Sample Collected

[a] Exact collection time will be decided after reviewing the PK data obtained on Cycle 1-Day 8

Table 11: USL311 PK Sample Times –Parts 3 & 4: Pre-Surgical USL311 Dosing

Day	Sample	Nominal Time[a,b]	Acceptable Collection Window
Pre-surgical Dosing, Day 1	1	Predose	≤ 24 hr prior to USL311 administration
	2	0.5 hr	± 2 min
	3	1 hr	± 5 min
	4	1.5 hr	± 5 min
	5	2 hr	± 5 min
	6	2.5 hr	± 5 min
	7	3 hr	± 5 min
	8	4 hr	± 5 min
	9	6 hr	± 5 min
Pre-surgical Dosing, Day 2	10	24 hr	± 60 min
Pre-surgical Dosing, Day 3[d]	11	60 hr	≥ 48 hr to < 72 hr[b,c]
Pre-surgical Dosing, Day 5[d]	12	108 hr	≥ 96 hr to < 120 hr[b,c]
Pre-surgical Dosing, Day 8[d]	13	Predose/Trough	≤ 30 min prior to USL311 administration
Day of Surgery	14	Pre-surgery	≤ 2 hr prior to surgery

[a] Pre-surgical Dosing PK samples are collected only for subjects randomized to receive USL311 prior to surgery. For subjects randomized to no USL311 treatment prior to surgery, PK samples will be collected starting at Cycle 1 post-surgery (see Table 12).
[b] All nominal times are relative to the start of USL311 infusion
[c] One sample to be collected anytime within the specified time window
[d] Samples not required if surgery occurs prior to the day of collection

Table 12: USL311 and Lomustine PK Sample Times – Parts 3 & 4: Dose-Expansion Post-Surgery Cycle 1

Day	Sample	Nominal Time (USL311, Parts 3 and 4)[a,b]	Nominal Time (Lomustine, Part 4 only)[c,d]	Acceptable Collection Window
Cycle 1-Day 1..	1	Predose/Trough	NS	≤ 30 min prior to USL311 administration
	2	0.5 hr	NS	± 5 min
	3	1 hr	NS	
	4	1.5 hr	NS	
	5	2 hr	NS	
	6	2.5 hr	NS	
	7	3 hr	NS	
	8	4 hr	NS	
	9	5 hr	NS	
	10	7 hr	NS	
Cycle 1-Day 2	11	24 hr	Predose	≤ 30 min prior to lomustine administration
	12	NS	1 hr	± 5 min
	13	NS	2 hr	
	14	NS	3 hr	
	15	NS	4 hr	
	16	NS	5 hr	
	17	NS	8 hr	
	18	NS	12 hr	
Cycle 1-Day 8	19	Predose/Trough	NS	≤ 30 min prior to USL311 administration
Cycle 1-Day 15	20	Predose/Trough	NS	≤ 30 min prior to USL311 administration
Cycle 2-Day 1	21	Predose/Trough	NS	≤ 30 min prior to USL311 administration

NS = No Sample Collected
[a] Samples to be collected from all subjects in Parts 3 and 4 regardless of participation in Pre-surgical Cycle
[b] Nominal times are relative to the start of USL311 infusion
[c] Lomustine samples collected only for subjects in Part 4 (GBM Dose-Expansion USL311 + lomustine)
[d] Nominal times are relative to lomustine dose administration

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Abbreviation	Definition
4-AP	4-aminopyridine
ADL	Activities of daily living
ADME	Absorption, distribution, metabolism and excretion
AE	Adverse event
AMD3100	Known antagonist of CXCR4, aka Plerixafor
ALC	Absolute lymphocyte count
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ANOVA	Analysis of variance
aPTT	Activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{0-t}	Area under the concentration-time curve from 0 to a specified time point
AUC_{0-tau}	Area under the concentration-time curve over a dosing interval
$AUC_{0-\infty}$	Area under the concentration-time curve from time 0 extrapolated to infinity
AUC % Extrapolated	Percent of $AUC_{0-\infty}$ due to extrapolation
AUMC	Area under the moment curve
β-hCG	Beta-human chorionic gonadotropin
Beclin-1	Protein which participates in the regulation of autophagy
BICR	Blinded independent central review
BMI	Body mass index
BMP	Di-docosahexaenoyl (22:6)-bis(monoacylglycerol) phosphate, biomarker of phospholipidosis
BSA	Body surface area
BUN	Blood urea nitrogen
C_0	Estimated plasma concentration at time 0
cAMP	Cyclic adenosine monophosphate
CCNU	1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; lomustine
CD11b	Leukocyte surface antigen CD11b

Abbreviation	Definition
CD133	Cancer stem cell (as well as other cells) antigen CD133
CD34+	Hematopoietic progenitor cell antigen CD34
CDER	Center for Drug Evaluation and Research
CES	Carboxylesterase
CFR	Code of Federal Regulations
CI	Confidence interval
CL	Systemic clearance
CL_{ss}	Steady-state systemic clearance
C_{avg}	Average concentration over a steady state dosing interval
C_{max}	Maximum plasma concentration
C_{min}	Minimum observed plasma concentration, occurring at tmin
CNS	Central nervous system
CPK	Creatine phosphokinase
CRA	Clinical research associate
CrCL	Creatinine clearance
CRO	Contract research organization
CSCs	Cancer stem cells
CSF	Cerebrospinal fluid
CSR	Clinical study report
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CV%	Coefficient of variation
CXCL12	C-X-C motif chemokine 12, ligand for CXCR4 (also known as SDF-1)
CXCR4	Chemokine (C-X-C Motif) receptor 4, an α-chemokine receptor in the GPCR gene family expressed by cells in the immune system and the central nervous system
CYP2D6	Cytochrome P450 2D6
CYP3A4	Cytochrome P450 3A4
CYP450	Cytochrome P450
DCR	Disease control rate
DEC	Dose Escalation Committee
DIPL	Drug-induced phospholipidosis
DLT	Dose-limiting toxicity
DSMB	Data Safety and Monitoring Board

Abbreviation	Definition
ECG	Electrocardiogram
EDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
EORTC QLQ-C30/BN20	European Organization for Research and Treatment of Cancer quality-of-life questionnaire, supplemented with brain cancer module
eCRF/CRF	Electronic case report form/ case report form
EIAED	Enzyme-inducing anti-epileptic drug
EU	European Union
EudraCT	EU Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
FLIPR	Fluorescent imaging plate reader
FI	Fluctuation index
FSH	Follicle-stimulating hormone
GBM	Glioblastoma multiforme
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GCPR	G-Coupled Protein Receptor
GGT	Gamma-glutamyl transpeptidase
GLP	Good Laboratory Practice
GM-CSF	Granulocyte macrophage colony stimulating factor
hCXCR4	Human chemokine (C-X-C Motif) receptor 4
Hct	Hematocrit
hERG	Human Ether-à-go-go-Related Gene
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HNSTD	Highest non-severely toxic dose
HRT	Hormone replacement therapy
IB	Investigator's Brochure
IC_{50}	Concentration of an inhibitor which causes the response (or binding) to be reduced by half
ICF	Informed consent form
ICH	International Conference on Harmonization
IDH1	Isocitrate dehydrogenase 1

Abbreviation	Definition
IEC	Independent Ethics Committee
IND	Investigational New Drug (application)
INR	International normalized ratio
IP	Investigational product
IRB	Institutional review board
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
IV	Intravenous
K-M	Kaplan-Meier
KPS	Karnofsky Performance Status
λ_z	Terminal rate constant
LC3	Microtubule-associated protein 1A/1B-light chain 3
LC-MS/MS	Liquid chromatography – mass spectrometry/mass spectrometry
LDH	Lactate dehydrogenase
M10	Major metabolite of USL311
mCRM	Modified continual reassessment method
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MGMT	O(6)-methylguanine-DNA methyltransferase
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
Nestin	A protein marker of neural stem cells
NYHA	New York Heart Association
ORR%	Objective response rate
OS	Overall survival
pCXCR4	Phosphorylated chemokine (C-X-C Motif) receptor 4
PD	Pharmacodynamic
PET	Positron emission tomography
PFS	Progression free survival
PFS-6m	6-month progression free survival
PICC	Peripherally inserted central catheter

Abbreviation	Definition
PK	Pharmacokinetic
PRN	Pro re nata, as needed
PT	Prothrombin time
PTT	Partial thromboplastin time
PV	Pharmacovigilance
QA	Quality assurance
QOL	Quality of life
QT	The measure of time between the start of the Q wave and the end of a T wave in the heart's electrical cycle
QTc	The corrected measure of time between the start of the Q wave and the end of a T wave in the heart's electrical cycle
QTcF	Fridericia corrected QT interval
RANO	Response Assessment in Neuro-Oncology
RBC	Red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended phase 2 dose
RT	Radiotherapy
SAE	Serious adverse event
SAP	Statistical analysis plan
SDF-1	Stromal cell-derived factor 1, ligand for CXCR4 (also known as CXCL12)
STD 10	Severely toxic dose in 10% of animals
$t_{1/2}$	Terminal plasma half-life
t_{max}	Time point of observed Cmax
t_{min}	Time of minimum observed plasma concentration
t_{last}	Time of last measurable plasma concentration
TdP	Torsade de Pointes
TEAEs	Treatment emergent adverse events
TK	Toxicokinetics
TMZ	Temozolomide
US	United States
ULN	Upper limit of normal
USL311	6-{4-[1-(propan-2-yl)piperidin-4-yl]-1,4-diazepan-1-yl}-N-(pyridin-4-yl)pyridine-2-carboxamide

Abbreviation	Definition
Vss	Volume of distribution at steady state
WBC	White blood cell
WOCBP	Women of child bearing potential

1. INTRODUCTION

1.1. Background Information

1.1.1. Disease Background

Glioblastoma multiforme (GBM) is a primary brain neoplasm, consisting of a genetically and phenotypically heterogeneous group of tumors (1, 2). Worldwide, there are an estimated 240,000 cases of brain and nervous system tumors per year and GBM has the worst prognosis (3). Moreover, approximately 10,000 – 14,000 patients are diagnosed with GBM each year in the United States (4). Despite aggressive treatment including surgical resection, chemotherapy and radiotherapy, median survival time for patients with GBM is reported to be only 15.7 months (5). The tumor almost invariably leads to neurological compromise and death. Due to its high degree of invasiveness, radical resection of the primary tumor mass is rarely curative. Infiltrating tumor cells invariably remain within the surrounding brain, leading to disease progression or recurrence, either locally or distant from the primary tumor (6). Recurrent disease occurs in more than 90% of patients and less than 5% of the patients are alive at 5 years (7).

The disease is thought to derive from a subpopulation of highly tumorigenic cells: glioblastoma stem cells which have a high propensity for recurrence (8). This hypothesis is supported by substantial experimental evidence (9-12). GBM stem cells, unlike the majority of the rapidly dividing tumor cells, are considered relatively quiescent. This renders them more resistant to conventional chemotherapy and radiotherapy (10). While most differentiated cancer cells die from chemotherapy and radiotherapy, cancer stem cells (CSCs) are believed to be triggered and repopulate to form recurrent tumors. CSCs are considered the primary source of invasion. Therefore, there is a clear need for novel treatment options which target the CSCs.

Standard-of-care for patients with newly diagnosed GBM is a surgical resection, if feasible, followed by radiation and temozolomide (Temodar®; TMZ). Maximal surgical resection is desired but this needs to be consistent with preservation of neurologic function, and in some patients, such as those who have disease that is not amenable to surgery or who have poor overall clinical condition, the best that can be achieved is a biopsy. Adjuvant radiotherapy improves local control and survival (13, 14) and adjuvant chemotherapy with TMZ improves progression free and overall survival (15, 16), although the effect is somewhat modest. However, in almost every case the disease will relapse.

Many of these patients with relapsed/recurrent GBM have significant tumor related symptoms and morbidity. Treatment is given with palliative intent and all treatments are associated with side effects. The mainstay of management of these patients with recurrent disease is systemic chemotherapy. Only 20 to 30% of patients may be eligible for a second resection (7) and survival ranges up to 36 weeks (17, 18). There is no evidence that patients treated with surgery have better outcomes that those given chemotherapy in this setting. The role of re-irradiation is also uncertain given that most patients will have already received maximal doses of conventional radiotherapy and prospective randomized trials are lacking. However, selected patients with good performance status may benefit from high precision radiation techniques such as stereotactic radiosurgery (19). Chemotherapy options for recurrent disease include bevacizumab and nitrosoureas. In Phase 3 t rials where lomustine (1-(2-chloroethyl)-3-

cyclohexyl-1-nitrosourea; CCNU) was the comparator arm, progression free survival and overall survival were reported as 2.7 and 9.8 months respectively in one trial (20) and 1.6 and 7.1 months respectively in another trial (21). The most important determinant of outcome in these patients is pre-treatment performance status.

The failure of conventional treatments and consequent poor prognosis highlights the clear unmet medical need for novel treatment options for patients with recurrent GBM after first line therapy. Given the central role of CSCs in GBM recurrence, treatment modalities that target CSCs within the central nervous system (CNS) may prove beneficial in the setting of recurrent GBM.

1.1.2. USL311 Background

USL311 (6-{4-[1-(propan-2-yl) piperidin-4-yl]-1,4-diazepan-1-yl}-N-(pyridin-4-yl) pyridine-2-carboxamide) is a selective antagonist of the chemokine receptor, C-X-C motif receptor 4 (CXCR4). CXCR4 is a G-coupled protein receptor (GCPR) whose natural endogenous ligand is the cytokine stromal cell derived factor 1 (SDF-1) also known as C-X-C motif chemokine 12 (CXCL12). CXCR4 manipulation, in combination with granulocyte colony stimulating factor (G-CSF), has proven to improve the outcome of hematopoietic (22) and endothelial progenitor (23) stem cell mobilization.

Recently, several studies have demonstrated the existence of CSCs for a variety of different tumor types, which constitute a reservoir of self-sustaining tumor cells with the ability to maintain tumor growth. Many of these cells express CXCR4 and respond to SDF-1, suggesting that CSCs represent a subpopulation capable of initiating metastasis in response to CXCR4 activation (24). There is also compelling evidence for a prominent role of CXCR4 in the establishment and protection of cancer stem-like niches in the bone marrow, which protect such cells from chemotherapy resulting in high relapse rates in some hematopoietic cancers (25). Additionally, SDF-1 is highly expressed in internal organs that represent the primary metastatic destinations of the corresponding cancer cells (26). Thus, CXCR4 overexpression by tumor cells makes them responsive to chemotactic gradients of SDF-1 and thereby promotes metastasis.

CXCR4 is one of the most common chemokine receptors that is overexpressed in over 23 different cancers (27). Published data on m alignancies with overexpression of CXCR4 include, but are not limited to: gliomas, neuroblastomas, astrocytomas, cervical, colorectal, thyroid, oral, esophageal, breast, ovarian, melanoma, prostate, lung, liver, renal, soft-tissue sarcomas and pancreatic cancer (27, 28). Moreover, CXCR4 overexpression correlates with poor prognosis in many types of cancer including, but not limited to: GBM (29), renal cell carcinoma (30), hepatocellular carcinoma (31), colorectal cancer (32), non-small cell lung cancer (33, 34), breast cancer (35), esophageal cancer (36) and prostate cancer (37). Due to the correlation of CXCR4 overexpression with a poor prognosis and also the involvement of CXCR4 in metastasis, CXCR4 is hypothesized to be a potential therapeutic target for a wide variety of cancers.

In GBM specifically, a strong correlation between CXCR4 expression and increasing histologic grade was observed in GBM tissue samples. In GBM patients, CXCR4 expression was associated with increased extent and intensity of T2-weighted perilesional magnetic

resonance imaging (MRI) signal abnormalities (38, 39). These findings suggest that increased CXCR4 expression correlates with increased tumor grade. Blockade of CXCR4 prevents both the vasculogenesis and recurrence of GBM tumors after radiation treatment in animal models (40). CXCR4 expression on CSCs is implicated in migration, self-renewal, as well as resistance to chemotherapy/radiotherapy and thus CXCR4 antagonism is expected to limit these processes and render CSCs more sensitive to concurrent therapies. In a human GBM cell line, administration of a CXCR4 antagonist (AMD3100, plerixafor) induced a significant increase in apoptosis (41). Similarly, in animal xenograft experiments, plerixafor significantly inhibited growth of the tumors by increasing apoptosis and decreasing proliferation of the tumor cells (42, 43).

These data from the literature provide the rationale for investigating USL311 as a potential anticancer agent for the treatment of solid tumors, including GBM.

1.2. Nonclinical Study Findings for USL311

A brief summary of the nonclinical study findings are provided here; for more information, please refer to the Investigator's Brochure (IB).

1.2.1. Primary Pharmacology (Efficacy)

The nonclinical pharmacology and efficacy of USL311 was characterized using studies of CXCR4 receptor activity in various cancer cell lines and in vitro assays, and in vivo using xenograft tumor models of GBM.

1.2.1.1. In Vitro Studies

USL311 is a potent antagonist of CXCR4 in mouse, rat, and human, with half maximal inhibitory concentrations [IC_{50}] shown to be 5.4, 5.8, and 1.1 nM [2.3, 2.4 and 0.46 ng/mL], respectively, in several in vitro studies. In these studies, cellular (blockade of cyclic adenosine monophosphate [cAMP] and β-arrestin readouts using human and mouse receptors) and cell-mediated (inhibition of SDF-1-induced chemotaxis) responses were blocked by incubation with USL311 in a concentration-dependent manner. The antagonism was shown to be highly selective for CXCR4 when measured against 19 separate chemokine receptors, 71 other receptors and receptor classes, and 27 enzyme systems, with minimal to no activity at other receptors.

1.2.1.2. In Vivo Studies

Numerous experiments were designed to assess the effect of USL311 (50 mg/kg/day orally, 5 days out of 7 continuously) either alone or in combination with anti-angiogenic chemotherapy (bevacizumab and sunitinib) as well as cytotoxic chemotherapy (TMZ) and/or radiotherapy (RT) on the growth of subcutaneous xenograft tumors in nude mice. As compared to vehicle, single-agent USL311 significantly inhibited the growth of T98G-, U251-, and U87MG-derived tumors induced in nude mice ($p < 0.001$). The delay in tumor growth with single-agent USL311 administration was similar to that observed with single-agent treatment with the comparators in these models (i.e., bevacizumab, sunitinib, RT, TMZ, and RT + TMZ).

In combination with the anti-angiogenic therapeutics, bevacizumab or sunitinib, USL311 inhibited the regrowth of T98G-, U251-, and U87MG-derived tumors in nude mice. Both USL311 and bevacizumab (4 mg/kg IV every 4 days, 8 injections total) were found to delay tumor growth in all three GBM cell line-derived xenografts tested, and the combination delayed progression significantly more than either agent alone ($p \leq 0.0001$). Similar results were observed when USL311 was combined with sunitinib (40 mg/kg orally daily x 3 weeks), where both USL311 and sunitinib delayed tumor growth in all three GBM cell line-derived xenografts tested and the combination of USL311 + sunitinib delayed progression significantly more than either agent alone for all three tumor types ($p \leq 0.0001$). The greater effect of USL311 in combination with either bevacizumab or sunitinib suggests that USL311 may potentiate the efficacy with anti-angiogenic chemotherapy.

Similarly, when combined with RT (2 Gy/every two days x 3 cycles), TMZ (16 mg/kg x 5 days orally) or RT + TMZ, USL311 significantly inhibited the regrowth of T98G-, U251-, and U87MG-derived tumors induced in nude mice. The delay in tumor regrowth was significantly greater for the combination of USL311 with RT, TMZ, and RT + TMZ as compared to treatment with RT, TMZ, or RT + TMZ without USL311, including in the T98G cell line which is resistant to TMZ (as it has an unmethylated O(6)-methylguanine-DNA methyltranferase [MGMT] promoter and therefore high MGMT expression). When administered with TMZ or RT plus TMZ, USL311 can inhibit tumor growth by 90%. The USL311-based combinations demonstrated an apparently synergistic effect (combination index = 0.39 – 0.75 with values <1 indicating synergy), suggesting that USL311 may potentiate the efficacy of RT (i.e., serve as a radiosensitizer) and cytotoxic chemotherapy regimens. In the latter case, the effect may conceivably be enhanced through continued dosing of a cytotoxic with USL311 as administration of TMZ was limited to the first 5 days of the experiment.

In summary, single-agent USL311 inhibited the growth of all GBM cell line-derived tumors as well as tumor regrowth when administered in combination bevacizumab, sunitinib, RT, TMZ, and RT + TMZ, with resultant increases in mouse survival.

1.2.2. Secondary Pharmacology

1.2.2.1. In vivo mobilization of hematopoietic progenitors and WBCs by USL311

In addition to the proposed anti-cancer effects, a key PD effect of CXCR4 manipulation is the mobilization of hematopoietic stem cells and white blood cells (WBCs) from the bone marrow to the bloodstream. The CXCR4 targeting agent, plerixafor (AMD3100; Mozobil®), is used clinically in combination with granulocyte-colony stimulating factor (G-CSF) to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation. The ability of single-dose USL311 (30 mg/kg subcutaneously) to induce in vivo mobilization of hematopoietic progenitors and WBCs from bone marrow in mice was investigated both alone and in combination with G-CSF (5 µg/day x 2 days).

Compared to vehicle, statistically significant increases in WBC count (1.7- to 1.9-fold, $p < 0.05$) were observed following dosing with USL311 as compared to vehicle, which was further increased in the presence of G-CSF (~3-fold). While both G-CSF and plerixafor (5 mg/kg subcutaneously) resulted in significant increases in hematopoietic progenitor cells in the blood compared to vehicle, no i ncrease was seen following single-agent USL311

administration. Both USL311 and plerixafor potentiated the effects of G-CSF on hematopoietic progenitor cell mobilization; however most of these findings were not statistically significant.

The difference in response to USL311 and plerixafor is attributed to differences in mechanism of action, as USL311 is a strict CXCR4 antagonist whereas plerixafor has been demonstrated to be a partial CXCR4 agonist. These results suggest that while increases in WBC count may be observed following USL311 administration, hematopoietic progenitor cells are not expected to be increased in the blood and thus maintained within the protective environment of the bone marrow when patients are dosed with USL311.

The ability of USL311 to increase the mobilization of WBCs and hematopoietic progenitor cells will be explored by measuring WBC count and CD34+ count (a marker of hematopoietic progenitor cells) at PK sampling matched time points. Additionally, SDF-1 levels may be measured at PK sampling matched time points, because increased SDF-1 levels are thought to be involved in the mobilization of hematopoietic progenitor cells (44) (see Section 8.7.3).

1.2.2.2. Effects of USL311 on angiogenesis in wound healing

Due to the involvement of the SDF-1/CXCR4 signaling pathway in angiogenesis, a study was conducted to assess the effect of USL311 on the progression of wound healing and specifically the process of wound angiogenesis. These effects were investigated in a study of wound healing in CD-1 mice using a model of standardized, full-thickness excisional wounds on the dorsal flanks of study animals. USL311 was administered orally at 10 or 50 m g/kg/day 24 hours prior to wounding which was followed, in a subset of animals, with daily dosing for 9 days post-wounding.

As compared to control (dosing vehicle), USL311 had no significant impact on overall wound closure, wound contraction, or the wound closure process of epithelialization. No significant effects on wound healing were observed upon histological evaluation. With regard to angiogenesis, in animals that received only a pre-wounding dose of USL311, angiogenesis was significantly increased by Day 10 post-wounding. In contrast, in animals that received both pre- and post-wounding USL311, a minor impairment of angiogenesis was observed by Day 10 post-wounding and did not increase with USL311 dose.

In conclusion, while USL311 showed small effects on wound angiogenesis, these effects were not sufficient to affect wound healing in the mouse and thus, it is expected that USL311 will not delay healing in patients who have recently undergone surgery.

1.2.3. USL311 Pharmacokinetics/Toxicokinetics

The PK and toxicokinetics (TK) of USL311 were investigated in a series of nonclinical studies addressing the absorption, distribution, metabolism, and excretion (ADME) of USL311 in mouse, rat, dog, and cynomolgus monkey. The following is a brief summary, please refer to the Investigator's Brochure for more information.

Following intravenous (IV) administration in mouse, rat, dog, and cynomolgus monkey, plasma USL311 concentrations exhibited a very rapid fall followed by a long, low terminal phase with a $t_{1/2}$ on the order of several days. In rat and dog studies, plasma USL311 PK appeared generally linear and dose-proportional increases in USL311 exposure were observed in plasma, brain and cerebrospinal fluid (CSF) samples. Consistent with the low plasma

USL311 concentrations exhibited during the terminal phase, minimal accumulation of USL311 was observed upon w eekly IV dosing. In contrast, tissue USL311 concentrations were significantly higher than those in the plasma, with tissue:plasma USL311 concentration ratios ranging from >100 (brain) to >10,000 (liver) with repeat IV or oral USL311 administration, consistent with the high volume of distribution across species. Following single-dose IV administration of radiolabeled USL311 to rats, significant uptake of radiolabeled drug was observed in most organs, including across the blood-brain barrier, as radioactivity was detectable in the brain up to 14 days post dose. This is consistent with estimated tissue USL311 half-lives on the order of 4 – 8 days. However, despite its long terminal half-life USL311 clearance is high, generally exceeding liver blood flow across species.

Plasma protein binding of USL311 is low, with in vitro estimates of USL311 fraction unbound in the plasma of 0.6 for rats and dogs and 0.8 for humans. As USL311 concentrations are higher in the whole blood versus the plasma (1.6 to 2.8-fold for humans), blood samples will be collected for analysis of USL311 PK in both the blood and plasma (see Section 8.6.1).

Metabolism of USL311 is attributed to both cytochrome P450 (CYP450) and CYP450-independent processes, specifically CYP3A4 and CYP2D6 and carboxylesterase activity (CES1 and CES2). In human hepatocyte experiments, the most abundant metabolites represented hydroxylation (M1) and dealkylation (M6 and M8) of USL311, as well as amide hydrolysis to carboxylic acid (M10) and 4-aminopyridine (4-AP, M26). The latter are the major metabolites observed following IV USL311 administration to rats, dogs and cynomolgus monkeys, with terminal half-lives similar to USL311. Plasma M10 exposures ranged from 2% (rat) to 120% (dog) of USL311 exposures and plasma 4-AP exposures were 3–20% of USL311 exposures in both species. In vitro studies suggest that formation of both M10 and 4-AP is primarily a CYP450-independent process, which has been attributed in part to CES1 and CES2 activity although CYP2D6 may also contribute. In contrast, CYP3A4 does not contribute to M10/4-AP formation but rather appears to be primarily responsible for the formation of a number of minor metabolites.

In vitro CYP450-interaction studies indicate that USL311 is unlikely to inhibit CYP1A2, 2C8, 2C9 or 2C19. USL311 displayed competitive inhibition of both CYP2D6 and CYP3A4 at high concentrations (IC_{50} > 10 µM [4.22 µg/mL]), although the latter appears to be sub-site specific as inhibition was only observed with midazolam as a substrate and not with testosterone. No time-dependent CYP450 inhibition was observed. In human hepatocytes, USL311 (up to 60 µM [25.3 µg/mL]) did not increase CYP1A2 or 2B6 activity, although a decrease in CYP2B6 activity was noted suggesting possible inhibition. USL311 did increase CYP3A4 activity by approximately 3-fold at 1 µM and 10 µM [0.42 and 4.22 µg/mL] concentration; however, this was not observed at the highest concentration (60 µM [25.3 µg/mL]) which is attributed to concomitant inhibition of CYP3A4 by USL311.

Studies in rats and dogs indicated that USL311 is excreted primarily via the biliary excretion in the feces. Excretion via the urine accounts for less than 2% of the total dose.

A summary of the mean plasma USL311 TK parameters from the pivotal rat and dog toxicology studies is shown in Table 13.

Table 13: Mean plasma USL311 TK parameters in rats and dogs (males and females combined) following weekly 2-hour IV infusions of USL311

Species	Study Day	Dose (mg/kg/ week)[a]	C_{max} (ng/mL)	AUC_{0-168} (ng.hr/ mL)	$AUC_{0-\infty}$ (ng hr/ mL)	CL (L/hr/ kg)	V_{ss} (L/kg)	Terminal $t_{1/2}$ (hr)
Rat	Day 1	15	258	1240	1260	11.9	269	31.1
		100	2510	13100	13800	7.25	248	48.7
		300/250[b]	11300	50200	55400	5.42	251	95.3
	Day 29	15	493	2350	--	--	--	--
		100	2730	19200	--	--	--	--
		300/250[b]	4510	57200	--	--	--	--
Dog	Day 1	5	65.7	1040	1260	4.10	375	86.2
		40	1420	11600	14100	2.92	261	106
		80	3460	23500	25900	3.12	242	99.6
	Day 29	5	84.2	1900	--	--	--	--
		40	2140	15300	--	--	--	--
		80	2150	25000	--	--	--	--

[a] Administered as a 2-hour IV infusion

[b] TK parameters for 300/250 mg/kg/dose group represent a 300 mg/kg dose on Day 1 and a 250 mg/kg dose on Day 29. Dose was decreased due to mortality in this treatment group.

1.2.3.1. USL311 Lysosomal trapping

The complex, multi-phasic PK profile and extensive tissue distribution exhibited by USL311 is attributed to sequestration within lysosomes (i.e., "lysosomal trapping"), cellular organelles that play a key role in various metabolic processes. USL311 has been shown to be lysosomotropic in vitro, and other lysosomotropic drugs exhibit similar PK and toxicology profiles as USL311, including a long terminal phase secondary to slow redistribution of intact drug from the lysosomes and a propensity for both drug-induced phospholipidosis and QTc prolongation (see Section 1.2.4). Consequently, it is presumed that despite the extensive tissue uptake a majority of USL311 may be lysosomally-trapped, as indicated by CSF concentrations (often used as a surrogate of unbound concentrations in the CNS) which were more than 100-fold lower than total brain tissue concentrations of USL311 in nonclinical studies. The unbound concentration of USL311 in the CNS will be assessed by collecting CSF in Phase 2 (see Section 8.6.2), which will provide information about the availability of study drug able to interact with the CXCR4 receptor in the CNS. However, it should be noted that lysosomal trapping may contribute to USL311's putative anti-cancer effect through inhibition of autophagy, a homeostatic cellular recycling process which is disrupted by other lysosomotropic drugs (e.g., chloroquine, hydroxychloroquine) leading to enhanced tumor cell death in vitro (45). The ability of USL311 to inhibit autophagy within tumor cells will be explored by measuring biomarkers of autophagy (e.g., LC3, Beclin-1) in resected tumor samples (see Section 8.7.2).

1.2.3.2. 4-Aminopyridine Metabolite

Based on in vitro and in vivo data, 4-AP is expected to be one of the primary metabolites of USL311 observed in humans. This metabolite is a broad-spectrum potassium channel blocker and is a US FDA-approved drug marketed under the name dalfampridine (Ampyra®) to improve walking in patients with multiple sclerosis. Of note, the prescribing information for dalfampridine carries a contraindication in patients with a history of seizures and a warning regarding an increased risk of seizures which may be increased in patients with moderate to severe renal impairment (creatinine clearance [CrCL] < 50 mL/min) (46). A plasma 4-AP concentration of 100 ng/mL is considered a likely threshold above which patients are at an increased risk of seizures in the absence of other risk factors (47). Relative to plasma USL311 concentrations, exposure to 4-AP was low in nonclinical studies following USL311 administration as a weekly 2-hour IV infusion, with mean plasma 4-AP concentrations < 35 ng/mL and < 75 ng/mL at the rat STD 10 (severely toxic dose in 10% of animals) and the dog HNSTD (highest non-severely toxic dose), respectively. As the starting USL311 dose of 60 mg/m^2 represents 1/10 of the rat STD 10, and subjects with renal insufficiency (CrCL < 60 mL/min) are excluded from participation, it is anticipated that plasma 4-AP concentrations will be low and will not exceed the 100 ng/mL threshold for increased risk of seizure.

Blood samples will be collected for evaluation of 4-AP PK and as data becomes available during study conduct, plasma 4-AP concentrations observed following USL311 administration will be considered for dose escalation decisions.

For more information on 4-AP, please refer to the prescribing information for dalfampridine (Ampyra®).

1.2.4. Toxicology and Safety Pharmacology

To establish the safety of USL311 the nonclinical package was designed in accordance with International Conference on Harmonization (ICH) S9 guidelines, and includes studies conducted via IV and oral administration. The nonclinical toxicology package includes:

- 4-week Good Laboratory Practice (GLP) toxicology assessments in rats and dogs incorporating safety pharmacology endpoints, urinary phospholipidosis biomarker, and toxicokinetic endpoints
- 7-day and 28-day exploratory repeated-dose studies in both rats and dogs
- Single-dose exploratory toxicology study using a single cardiovascular-telemetry implanted dog to confirm tolerated dose in a drug-naïve dog
- non-GLP Ames and mouse lymphoma screening assays
- non-GLP hERG assays for Safety Pharmacology
- GLP assessments of hemolysis and flocculation in vitro

Lethal doses were defined in both rats and dogs following IV and oral administration. In rats, mortality was observed following weekly IV administration (2-hour infusion) at doses of 300 mg/kg/dose and higher, and following oral daily dosing at 100 mg/kg/day and higher. In dogs, administration of USL311 was associated with mortality at 100 mg/kg and higher.

Minimal decreases in body weight (i.e., decreased weight gains) and food consumption have been observed at higher doses. Consistent across studies, administration of USL311 was associated with transient CNS-related clinical signs including, but not limited to, tremors, ataxia, unsteady gait, slow deliberate movement, lethargy, semi-closed eyes, salivation, vomiting, and diarrhea on the day of infusion. Functional observational battery assessments on Days 9 and 23 of the rat GLP toxicology study found no evidence of lasting neurotoxicity. Likewise, there was no evidence of neuropathology in the rat or dog toxicology studies.

A possible interaction of USL311 with anesthesia administration was uncovered in the dog GLP toxicology study during the CSF collection on Day 15 that occurred within 1 hour after the end of the 2-hour USL311 infusion. Male and female dogs given the highest USL311 dose (80 mg/kg/dose) exhibited twitching and kicking following IV dexmedetomidine administration, with the effect being more severe in the female dogs. For the female dogs, additional IV dexmedetomidine was given to reduce movement and ensure full sedation for the CSF collection. This effect appears to be acute as it was only observed when the dogs were immediately anesthetized following USL311 infusion. When approximately 24 hours intervened between the final USL311 infusion and anesthesia, the effect was not observed. The proposed mechanism for the interaction is that greater concentrations of 4-AP were formed at the higher doses administered to the dogs demonstrating the possible interaction. 4-AP acts as a neuroexcitatory agent through blockade of potassium channels, as well as through multiple other mechanisms, while systemic anesthetics act as neuroinhibitory agents through a variety of different mechanisms, depending on the agent used. Thus, the interaction occurs due to opposing pharmacological mechanisms which only appear to occur at high doses of USL311 resulting in high concentrations of 4-AP.

In vitro hERG cardiac channel assays found a relatively low IC_{50} of 1.5–2.6 µM. ECG assessments in the dog toxicology studies (collected from 3 hours from the start of infusion) demonstrated QT prolongation with up to a 12% increase in Fridericia corrected QT interval (QTcF) relative to time-matched control following IV doses of USL311 of 40 mg/kg and higher. These effects (also seen in exploratory toxicology studies) were found to be correlated with plasma USL311 concentrations such that the QTcF interval decreased following the end of the 2-hour infusion and a return to near baseline was noted by approximately 12-20 hrs post dose. The prolongation in QTcF attenuated with repeated dosing, as the largest effect on QTcF interval was observed following the first dose of USL311. Increased heart rates were also noted at USL311 doses of 40 mg/kg and higher, but the primary effect on heart rate in the dog GLP toxicology study was lower heart rates following infusion with repeated dosing. The no-observed effect level (NOEL) for QTcF prolongation and heart rate effects was 5 mg/kg in the pivotal dog IV toxicology study, corresponding to a human equivalent dose of 100 mg/m^2 which is higher than the 60 mg/m^2 starting IV dose.

For both species, clinical pathology changes including a 2-fold increase in AST have been observed. Increases in WBC counts have been observed and are expected pharmacology for USL311 (see Section 1.2.2.1). With the higher doses in the pivotal rat GLP toxicology study, some mild changes in RBC parameters (including decreased MCV and MCH and increased RBC count) were observed, increased inorganic phosphorus (males only), increased urine volume with decreased specific gravity (females only), and increased urine magnesium:urine creatinine ratio with no correlative changes in serum magnesium or calcium. In the pivotal dog

GLP toxicology, a shorter aPTT was observed with higher doses of USL311 in female dogs. Histologically, treatment with USL311 produced microscopic changes consistent with phospholipidosis in a number of tissues. Dose- and time-related vacuolar/foamy changes in phagocytic and epithelial cells were also consistent with intracellular accumulation of USL311, most likely within lysosomes (see Section 1.2.3.1), and with recruitment plus proliferation of phagocytic and inflammatory cells. With once weekly IV dosing in rats, minimal degeneration and necrosis of skeletal muscle have been observed with doses at or above the STD 10. Dose related increases in the urinary BMP phospholipidosis biomarker have been observed in the rat and dog toxicology studies to support the microscopic findings indicative of drug-induced phospholipidosis.

Acceptable local tolerance has been demonstrated in the GLP toxicology studies in the catheterized animals. In vitro hemolysis and flocculation assays indicate that USL311 will be compatible with blood when administered at physiologically relevant concentrations, and there was no evidence of hemolysis observed in any of the toxicology studies conducted in rats and dogs. Further, Ames and mouse lymphoma screening assays indicate that USL311 is not mutagenic.

The nonclinical studies conducted to date support the use of USL311 in the clinic and based on both rat and dog findings, an adequate safety margin exists to initiate first in human dosing. Calculations based on the algorithm for first-in-human dose selection in the ICH S9 guidance demonstrate that the rat STD 10 provides the most conservative clinical starting dose (Table 14) for the IV dose in Part 1a.

Table 14: Clinical Starting Dose Estimated from GLP Toxicology Studies

Based on Pivotal GLP Toxicology Studies		Human Equivalent Dose[a]	Clinical Start Dose per ICH S9 Algorithms
Rats	STD 10 = 100 mg/kg/dose	600 mg/m^2	1/10 STD 10 = 60 mg/m^2
Dogs	HNSTD = 80 mg/kg/dose	1600 mg/m^2	1/6 HNSTD = 267 mg/m^2

[a] Human Equivalent Dose calculations are based on body surface scaling per the U.S. FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, CDER, July 2005

Part 1b of the study will resume under this amendment with dosing initiation of an oral formulation of USL311, at a starting dose of 40 mg once daily in 3-week (21-day) cycles. The selected 40 mg daily oral starting dose is comparable to the 30 mg daily starting dose based on 1/6 HNSTD in dogs (5 mg/kg/day oral daily dose) and is lower than the ~60 mg daily dose calculated based on the maximum IV dose tested in humans (250 mg/m2 weekly, divided into daily doses). USL311 plasma pharmacokinetics are dose-proportional and minimal to no plasma accumulation is expected for USL311 and its main metabolites after daily oral dosing. In addition, a fixed-dosing approach is considered to be appropriate for future studies given that USL311 pharmacokinetics are not influenced by body size. Daily oral dosing is anticipated to further mitigate the concentration-related QT effect, compared to IV bolus weekly dosing.

1.3. Clinical Study Findings for USL311

There are no completed clinical trials of USL311. This study represents the first-in-human study of USL311.

As of the date of this amendment, data from 13 subjects participating in the Part 1a IV Dose Escalation portion of this study have been collected at USL311 doses of 60, 120, 180, and 250 mg/m^2 via 2- or 4-hour IV infusion. Interim data from the first 8 subjects suggested a potential USL311-related increase in the QT interval, which was transient, resolved post-end of infusion, and exhibited an apparent correlation with plasma USL311 concentrations (i.e., C_{max}). Based on these observations, a longer duration of USL311 infusion (i.e., slower infusion rate) was expected to decrease the magnitude of QT-prolongation and, therefore; the infusion duration was increased from 120 minutes (2 hours) to 240 minutes (4 hours) in Amendment #3. Dosing with the 4-hour infusion was initiated at the highest safe dose of USL311 as determined from subjects who received USL311 via a 2-hour infusion (i.e., prior to Amendment #3). Subjects who were previously enrolled and were actively participating at the time Protocol Amendment #3 was activated would continue study participation according to Protocol Amendment #2.

After 13 subjects were evaluable (i.e., had completed Cycle 1 dosing, at minimum) in Part 1a IV Dose Escalation, the Dose Escalation Committee halted dose escalation, although no DLTs had occurred, due to continuing dose-related increases in QTcF prolongation that was not ameliorated by increasing the infusion duration. Part 1b of the study will resume under this amendment with dosing initiation of an oral formulation of USL311, at a starting dose of 40 mg once daily in 3-week (21-day) cycles. The selected 40 mg daily oral starting dose is comparable to the 30 mg daily starting dose based on 1/6 HNSTD in dogs (5 mg/kg/day oral daily dose) and is lower than the ~60 mg daily dose calculated based on the maximum IV dose tested in humans (250 mg/m^2 weekly, divided into daily doses). USL311 plasma pharmacokinetics are dose-proportional and minimal to no plasma accumulation is expected for USL311 and its main metabolites after daily oral dosing. In addition, a fixed-dosing approach is considered to be appropriate for future studies given that USL311 pharmacokinetics are not influenced by body size. Daily oral dosing is anticipated to further mitigate the concentration-related QT effect, compared to IV bolus weekly dosing.

As of the date of this amendment there were no active subjects currently participating in Part 1a IV Dose Escalation. All subsequent Part 1 participants will be enrolled in Part 1b, Oral Dose Escalation.

2. STUDY OBJECTIVES

2.1. Phase 1: Parts 1 and 2 Dose-Escalation in Subjects with Advanced Solid Tumors

2.1.1. Primary Objectives

The primary objectives of the Phase 1 dose-escalation are to:

- Determine the MTD and RP2D of USL311 as a single agent in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment (Part 1)
- Determine the MTD and RP2D of USL311 in combination with lomustine in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment (Part 2)

2.1.2. Secondary Objectives

The secondary objectives of the Phase 1 dose-escalation are to:

- Assess the safety and tolerability of USL311 as a single agent and in combination with lomustine
- Determine preliminary efficacy parameters such as PFS-6m, ORR%, DCR, PFS and OS of USL311 as a single agent and in combination with lomustine
- Determine the PK profile of USL311 in plasma and whole blood and of lomustine in plasma (prior to and with concomitant USL311 administration)
- Evaluate the drug interaction potential between USL311 and lomustine

2.1.3. Exploratory Objectives

The exploratory objectives of the Phase 1 dose-escalation are to:

- Determine effects of USL311 on systemic markers of CXCR4 inhibition, including measurement of CD34+ cells, and WBC count
- Measure effects of USL311 on a urine biomarker of phospholipidosis, BMP
- Investigate exposure-response relationships for USL311 as a single agent and in combination with lomustine

2.2. Phase 2: Parts 3 and 4 Dose-Expansion in Subjects with Relapsed/Recurrent GBM

2.2.1. Primary Objectives

The primary objectives of the Phase 2 dose-expansion are to:

- Determine the percentage PFS-6m of USL311 as a single agent in subjects with relapsed/recurrent GBM who previously received standard-of-care treatment in the first-line setting and who are candidates for re-resection (Part 3).
- Determine the percentage PFS-6m of USL311 in combination with lomustine in subjects with relapsed/recurrent GBM who previously received standard-of-care treatment in the first-line setting and who are candidates for re-resection (Part 4)

2.2.2. Secondary Objectives

The secondary objectives of the Phase 2 dose-expansion are to:

- Assess the safety and tolerability of USL311 as single agent and in combination with lomustine in subjects with relapsed/recurrent GBM
- Assess ORR%, PFS, DCR and OS of USL311 as single agent and in combination with lomustine in subjects with relapsed/recurrent GBM
- Determine the PK profile of USL311 in plasma and whole blood and of lomustine in plasma

2.2.3. Exploratory Objectives

The exploratory objectives of the Phase 2 dose-expansion are to:

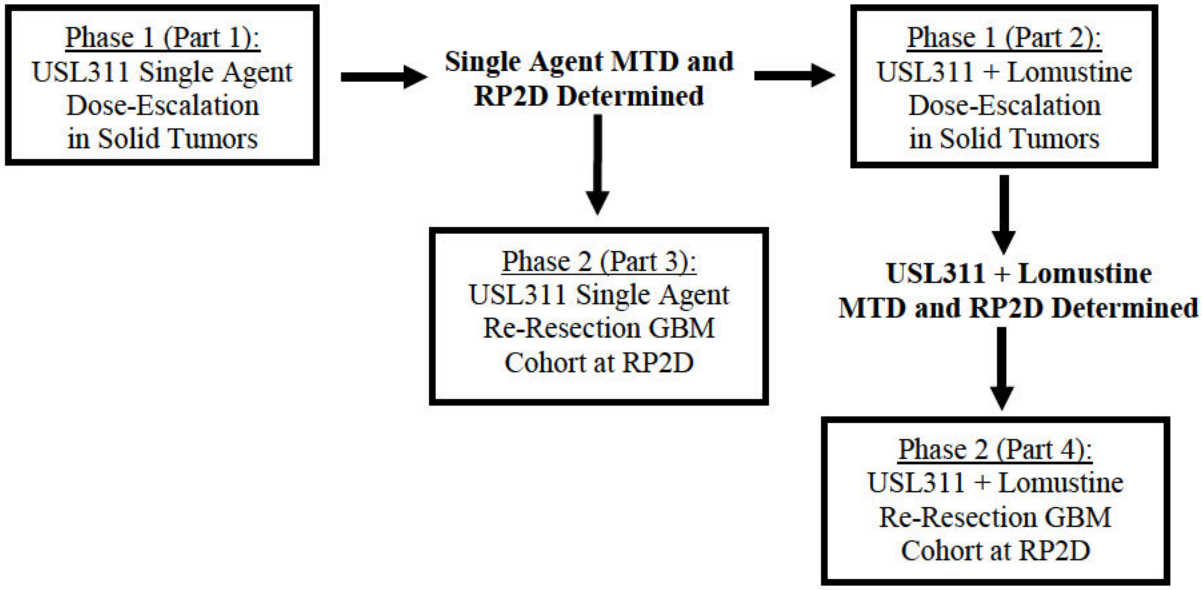
- Assess PD markers of CXCR4 inhibition in tumor samples following pre-surgical administration of USL311 for comparison with subjects randomized to receive no pre-surgical USL311 treatment. These markers may include, but are not limited to, the following:
  - Tumor pCXCR4 profile
  - Tumor markers of autophagy (e.g., LC3, Beclin-1)
  - Tumor markers of vasculogenesis (e.g., CD11b) and cancer stem cell survival and proliferation (e.g., nestin, CD133)
- Determine biodistribution of USL311 to the brain/CSF in resected tissue samples and CSF samples following pre-surgical administration of USL311 to investigate CNS penetration and for correlation with clinical response and/or PD data
- Assess exposure-response relationships for USL311 as a single agent and in combination with lomustine
- Assess health-related QOL and subject reported outcomes (EORTC QLQ-C30/BN20)

3. INVESTIGATIONAL PLAN

3.1. Overview

This is a Phase 1/2 open-label, multicenter study in four parts: 1) single agent USL311 (IV and oral) dose escalation in subjects with advanced solid tumors, 2) dose escalation of oral USL311 in combination with lomustine in subjects with advanced solid tumors, 3) preliminary efficacy evaluation of single agent oral USL311 in subjects with relapsed/recurrent GBM who are candidates for re-resection, and 4) preliminary efficacy evaluation of oral USL311 in combination with lomustine in subjects with relapsed/recurrent GBM who are candidates for re-resection. Subjects will only participate in one part of the study. Parts 1 and 2 (Phase 1 component) will be conducted at approximately 4 study sites and Parts 3 and 4 (Phase 2 component) will be conducted at approximately 10 study sites.

Figure 1: Phase 1/2 Study Components Flow Diagram



3.2. Phase 1 – Dose-Escalation in Subjects with Advanced Solid Tumors

Dose-escalation components will 1) assess safety, PK/PD and preliminary efficacy of USL311 as a single agent in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment, and 2) assess safety, PK/PD and preliminary efficacy of the combination of USL311 with lomustine in subjects with advanced solid tumors for whom no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment.

The dose-escalation component will determine the MTD/RP2D of single-agent USL311 (Part 1) and USL311 in combination with lomustine (Part 2).

3.2.1. Part 1a – Dose-Escalation in Advanced Solid Tumors of Single Agent IV USL311

Subjects in Part 1awill participate in the following visits:

- Screening Visit (within 28 to 2 days of Cycle 1-Day 1)
- Baseline Visit (within 1 day of Cycle 1-Day 1)
- Treatment Phase Visits for Cycle 1 (Visits on Day 1, Day 2, Day 3, Day 5, Day 8, and Day 15)
- Treatment Phase Visits for Cycle 2+ (Visits on Day 1, Day 8, and Day 15)
- End of Treatment Visit (within 14 da ys of the decision to discontinue on-study treatment)
- Follow-up Visit (within 28 days of last dose of USL311; may be same visit as End of Treatment Visit)
- Long Term Follow-up Visits (quarterly visit or phone call until subject death or study termination)

During the IV Treatment Phase, treatment will be administered once weekly in 3-week (21-day) cycles. USL311 will initially be administered IV over 120 minutes (2 hours, Protocol Amendment #2) or 240 minutes (4 hours, Protocol Amendment #3) on Days 1, 8, and 15 of a 21-day cycle. Subjects will be monitored by study staff for at least 8 hours after the start of the infusion for their first dose of USL311.

Dosing with the 4 hou r infusion will be initiated at the highest safe dose of USL311 as determined from subjects who received USL311 via a 2 hour infusion (viz., prior to Amendment #3). Subjects who were previously enrolled and are actively participating at the time Protocol Amendment #3 is activated will continue study participation according to Protocol Amendment #2.

There re a total of 31 possible IV dose levels specified between 40 mg/m^2 and 1660 mg/m^2. Dose levels are defined to be in approximately 15% increments. Specifically, the dose levels in mg/m^2, are shown in Table 15 below:

Table 15: USL311 Available IV Doses for Dose Escalation

Available Doses (mg/m^2)			
40	120	320	820
50	140	360	920
60	160	400	1040
70	180	450	1170
80	200	510	1310
90	220	570	1480
100	250	650	1660
110	280	730	

Dose escalation will begin with the single agent with enrollment of subjects to the 60 mg/m^2 dose. A rationale for the selection of the starting dose and administration duration is provided in Section 6.1.2.1. Three subjects will be treated at the initial dose of 60 mg/m^2 and evaluated by the DEC, after all three subjects had completed Cycle 1, before the dose was escalated/de-escalated. The 40 and 50 mg/m^2 dose levels were de-escalation dose levels. Dose-escalation was to follow a modified continual reassessment method (mCRM) design. The dose escalation strategy is further described in Section 3.2.4.

For each dose escalation, one (1) subject will be enrolled at the new dose level and will be treated and observed from Cycle 1-Day 1 through Cycle 1-Day 8 for tolerance to the dose before additional subjects may be treated at that dose level. Details are provided in the Dose Escalation Management Plan.

At each dose-escalation point, the DEC (see Section 3.2.6) will review the safety data, any available PK/PD data, and all other relevant subject data in order to recommend the subsequent dose level **and IV infusion duration**. Clinical judgment will always supersede the model-based recommendations for dose-escalation, meaning that clinical judgment can be applied to reduce the increment of dose escalation or elect not to dose escalate in favor of accruing more subjects at that dose level.

Treatment cycles will be repeated every 21-days until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study.

3.2.2. Part 1b – Dose-Escalation in Advanced Solid Tumors of Single Agent Oral USL311

Subjects in Part 1b will participate in the following visits:

- Screening Visit (within 28 to 2 days of Cycle 1-Day 1)
- Baseline Visit (within 1 day of Cycle 1-Day 1)
- Treatment Phase Visits for Cycle 1 (Visits on Day 1, Day 2, Day 3, Day 5, Day 8, and Day 15)
- Treatment Phase Visits for Cycle 2+ (Visits on Day 1, Day 8, and Day 15)
- End of Treatment Visit (within 14 days of the decision to discontinue on-study treatment)
- Follow-up Visit (within 28 days of last dose of USL311; may be same visit as End of Treatment Visit)
- Long Term Follow-up Visits (quarterly visit or phone call until subject death or study termination)

Treatment will be administered once daily in 3-week (21-day) cycles. USL311 will be administered as oral tablets once daily in the morning under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 1, 2, 8, and 15 and on Cycle 2-Day 1. For all other outpatient dosing days the subject should be instructed to make an effort to take the

oral tablets at least 1 hour before or at least 1 hour after their meal, and they will be instructed to document in their diary the time of any food eaten prior to dosing and for 1 hour after dosing. It is recommended that subjects take their dose at the same time each day, except for visit days, when the dose will be held and taken during the clinic visit. If a dose is missed in the morning, and fewer than 12 hours have elapsed since the planned administration time, the subject should take the missed dose. If more than 12 hours have elapsed since the planned administration time, the missed dose should be skipped. A diary will be provided to the subject to document the date and time of each daily dose and the time of any food eaten prior to dosing and for 1 hour after dosing.

There is a total of 27 possible dose levels specified between 20 mg and 1440 mg. Dose levels are defined to be in approximately 15% increments, and rounded down to the nearest multiple of 20 in order to accommodate dosing with the available 20 mg and 100 mg strengths of the USL311 oral formulation. The dose levels in mg, are shown in Table 16, below:

Table 16: USL311 Available Oral Doses for Dose-Escalation

Available Doses (mg)			
20	180	400	1100
40	200	460	1260
60	220	520	1440
80	240	580	
100	260	660	
120	280	740	
140	320	840	
160	360	960	

Dose escalation will begin in the single agent oral formulation with enrollment of subjects to the 40 mg dose. A rationale for the selection of the starting dose and administration duration is provided in Section 6.1.2.1. The 20 mg dose level is a de-escalation dose level. Dose-escalation will follow a modified continual reassessment method (mCRM) design. The dose escalation strategy is further described in Section 3.2.4.

For each dose-escalation, one (1) subject will be enrolled at the new dose level and will be treated and observed from Cycle 1-Day 1 through Cycle 1-Day 8 for tolerance to the dose before additional subjects may be treated at that dose level. Details are provided in the Dose Escalation Management Plan.

At each dose-escalation point, the DEC (see Section 3.2.6) will review the safety data, any available PK/PD data, and all other relevant subject data in order to recommend the subsequent dose level. Clinical judgment will always supersede the model-based recommendations for dose-escalation, meaning that clinical judgment can be applied to reduce the increment of dose escalation or elect not to dose escalate in favor of accruing more subjects at that dose level.

3.2.3. Part 2 – Dose Escalation in Solid Tumors of Combination USL311 plus Lomustine

Once the MTD is determined for single agent USL311, dose-escalation of USL311 in combination with lomustine may begin. Subjects in Part 2 will participate in the following visits:

- Screening Visit (within 28 days of Cycle 1-Day 1)
- Treatment Phase Visits for Cycle 1 (Visits on Day 1, Day 8, Day 9, Day 15, Day 22, Day 29, Day 36, and Day 43)
- Treatment Phase Visits for Cycle 2+ (Visits on Day 1, Day 2, Day 8, Day 15, Day 22, Day 29, and Day 36)
- End of Treatment Visit (within 14 days of decision to discontinue on-study treatment)
- Follow-up Visit (within 28 days of last dose on USL311; may be same visit as End of Treatment Visit)
- Long Term Follow-up Visits (quarterly visit or phone call until subject death or study termination)

During the Treatment Phase, lomustine will be administered as a single oral dose every 7 weeks (during Cycle 1) and every 6 weeks for subsequent cycles (Cycle 2+) and USL311 will be administered as an oral tablet once daily during Cycle 1 starting on Day 8 or over the subsequent 6-week cycles (during Cycle 2+) starting on Day 1. To evaluate drug-drug interaction potential between USL311 and lomustine, the first dose of lomustine in Cycle 1 will be administered on Cycle 1-Day 1, one week prior to the first dose of USL311 (Day 8). All remaining doses of lomustine (Cycle 2+) will be administered on Day 2 of the cycle. USL311 will be administered daily, continuously as of Cycle 1-Day 8, with no break between cycles. Subjects will be monitored by study staff for at least 4 hours after their first dose of USL311.

This dose escalation for USL311 in combination with lomustine will also be conducted according to a mCRM model similar to that described for the single agent in Part 1. However, the dose range will be informed by results of USL311 as a single agent. The starting dose of USL311 when combined with lomustine will be at least two (2) evaluated dose levels below the MTD determined for USL311 as a single agent and the starting dose of lomustine will be 90 mg/m^2. A dose de-escalation for lomustine to 80 mg/m^2 may occur depending on the tolerability of the initial lomustine dose. If USL311 is escalated in combination with lomustine to the same dose level as the single agent MTD, no further escalation of USL311 will be allowed. However, subsequent subjects may be enrolled to evaluate dose escalation of lomustine up to a maximum of 130 mg/m^2 in combination with USL311 (dosed at the MTD). The dose escalation strategy is further described in Section 3.2.4.

For each dose-escalation, one (1) subject will be enrolled at the new dose level and will be treated and observed from Cycle 1-Day 1 through Cycle 1-Day 15 for tolerance to the combination dose before additional subjects may be treated at the dose level. Details are provided in the Dose Escalation Management Plan.

At each dose-escalation point, the DEC (see Section 3.2.6) will review the safety data, any available PK/PD data, and all other relevant subject data in order to recommend the subsequent dose level. Clinical judgment will always supersede the model-based recommendations for dose-escalation, meaning that clinical judgment can be applied to reduce the increment of dose escalation or elect not to dose escalate in favor of accruing more subjects at that dose level.

Treatment cycles beyond Cycle 1 will be repeated every 6 weeks (42-days) until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study. The MTD will be determined for USL311 in combination with lomustine, and all available safety, PK, and PD data will be reviewed by the DEC and Sponsor prior to determining the RP2D. The RP2D will be determined by the DEC and Sponsor after considering the safety, PK/PD, and preliminary efficacy outcomes and may be equal to or lower than the MTD.

3.2.4. Dose-Escalation Strategy

During Phase 1 dose escalation, subjects will receive study drug USL311 in Part 1 or USL311 in combination with lomustine in Part 2 at increasing doses. Decisions on dose-escalation will be made according to the mCRM design and reviewed by the DEC as described in Section 3.2.6. Complete details of the mCRM model and its operating characteristics for Part 1 are provided in Appendix 1a. An additional report containing the details of the mCRM model and its operating characteristics for Part 2 is described in Appendix 1b. The target dose-limiting toxicity (DLT) rate is 33%. The MTD will be defined as the highest safe dose, where safe is defined by having at least a 50% probability that the DLT rate is less than 33%. This definition for safety is consistent with the maximum likelihood estimate for the probability of DLT. If the DLT rate is estimated to be exactly 33%, the probability the DLT rate is less than 33% will be equal to 50%. Therefore, doses with a mean estimated DLT rate less than 33% will be considered safe by this definition and doses with a mean estimated DLT rate greater than 33% will be considered unsafe by this definition. Subjects may never be assigned to a dose that is considered unsafe according to this definition.

A dose-toxicity model will be used to determine which doses are safe and to assign subjects to doses, i.e. to assign subjects to the highest expected safe dose. However, assignment of dose levels is also governed by rules concerning the speed of dose escalation and rules that determine whether an untried dose level may be skipped. When the dose is escalated, it may only escalate to the highest safe dose that is no more than a 100% increase over the current dose level. If the dose must be de-escalated, the dose will de-escalate to the highest safe dose regardless of how large a decrease in dose level. As long as no DLT has yet been observed, there must be complete DLT information through Cycle 1 on at least 2 subjects in order to escalate. Once the first DLT is observed in the study (Part 1 and Part 2), there must be complete DLT information through Cycle 1 on at least 3 subjects in order to escalate.

In the dose-toxicity model, non-DLT toxicity events (i.e. grade 2 toxicities) will inform the probability of DLT at each dose level. The DLT observation period, for the purpose of dose-escalation, is one (1) cycle. Once a subject has completed this first cycle, DLT observation period, the subject will be considered to have complete DLT information for the purposes of making dose escalation decisions for the next subject enrolling into the study (Part 1 and Part 2). However, DLTs may also appear in later cycles. If a DLT appears at any time

through subsequent cycles of treatment, a subject's DLT status and mCRM model will be updated to reflect the late cycle DLT.

The first three subjects will be enrolled as a dosing cohort in Part 1 (and possibly for Part 2). There must be complete DLT information on these three subjects in order to enroll the 4th subject. Starting with the 4th subject enrolled, there will be open enrollment to the study meaning that subjects can be enrolled as they become available for the study, however, generally, there may be no more than 3 subjects enrolled in the study (Part 1 and Part 2) with unknown DLT information at any time.

This study will be monitored for safety and for success in identifying the MTD. If no doses are safe, dose escalation will stop and no MTD will be declared. Alternatively, dose escalation may be stopped early when we are sufficiently confident the MTD has been identified. We characterize this by having either estimated the MTD with high probability, or by having a sufficient number of subjects with complete DLT information at and around the MTD. If the dose escalation is not stopped early for safety or for success in identifying the MTD it will continue to the maximum sample size of 40 subjects. Refer to Appendix 1b for more detailed information regarding the stopping rules and determination of the MTD for dose-escalation.

No intra-subject dose-escalation will be allowed until the MTD is established, at which time subjects who have received at least 12 weeks of study drug(s) without evidence of progression may receive study drug up to the RP2D, at the discretion of the Investigator and with approval of the Sponsor.

3.2.5. Dose-Limiting Toxicity

A DLT will be defined as one or more of the following toxicities occurring during treatment during Phase 1 (Parts 1 and 2) dose-escalation. All toxicities are considered related to study drug (either USL311 and/or lomustine) unless the event is clearly and incontrovertibly due to another cause (e.g., a subject's underlying disease). Toxicities will be graded according to the NCI CTCAE version 4.03.

Hematologic Toxicity:

- Grade 4 neutropenia lasting > 7 days in the absence of growth factor support
- Grade 3 neutropenia of any duration associated with fever ≥ 38.5°C
- Grade 3 thrombocytopenia with bleeding
- Any other grade 4 hematologic toxicity

Non-hematologic Toxicity:

- Grade 3 nausea, vomiting, and/or diarrhea that lasts > 48 hours despite maximum medical support
- Grade 3 electrolyte imbalance that does not correct within 48 hours to < grade 2 despite maximal medical intervention
- Grade 3 fatigue that does not improve to ≤ grade 2 within 5 days

- Grade 3 QTcF interval prolongation with a > 60 ms change from baseline
  - For DLT assessment based on safety ECGs, baseline is defined as the last ECG collected prior to the first dose of study drug. For DLT assessment based on extracted ECGs, baseline is defined as the closest time-matched extracted ECG (triplicate average) from Cycle 1-Day -1 for Part 1 or the average of the Cycle 1-Day 1 ECG extractions for Part 2
- Any other grade ≥ 3 adverse event (AE) considered possibly related to study drug(s), except:
  - Non-hematologic laboratory grade 3 AE that is asymptomatic and/or rapidly reversible (returned to baseline or to grade ≤ 1 within 7 days) unless identified as clinically relevant by the Investigator

Other Toxicities:

- Any IV dose(s) or 7 consecutive oral doses missed within Cycle 1 due to a possible study drug(s)-related toxicity
- Inability to administer scheduled cycle of treatment within 21 days of the scheduled start of Cycle 2 due to a possible study drug(s)-related toxicity

3.2.6. Dose Escalation Committee

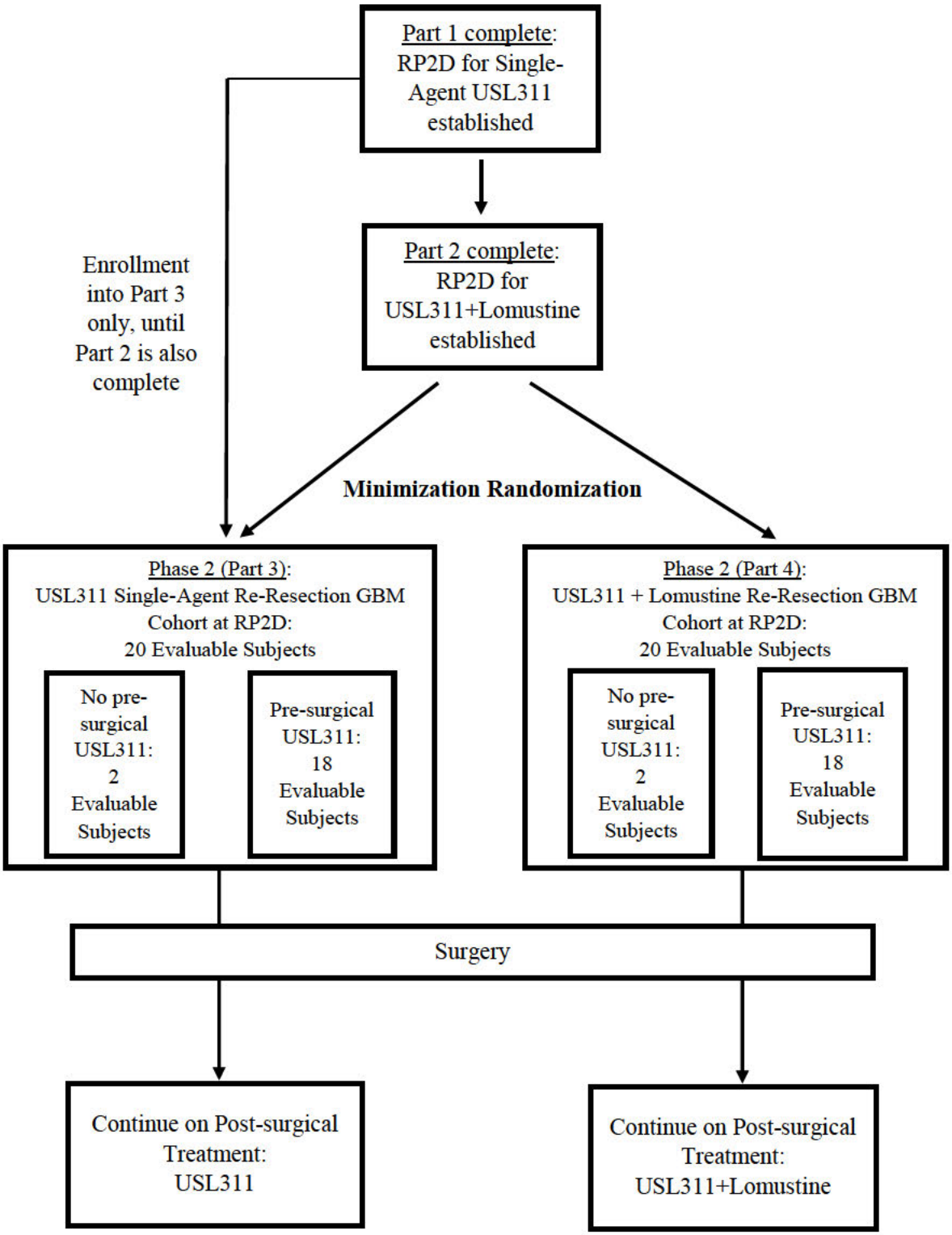
During dose-escalation for Phase 1 (Parts 1 and 2), the safety data, any available PK/PD data, and other relevant subject data will be reviewed by the DEC. The DEC will comprise, at a minimum, Sponsor Medical Monitor, Contract Research Organization (CRO) Medical Monitor and the study site Investigator(s) (or designee). Other representatives may be involved in data review and the dose-escalation decision, as appropriate. All dose-escalation steps and dose schedule and infusion duration, if applicable, recommendations will be agreed upon by the DEC before dosing at the next dose level or determining the MTD and RP2D. Detailed information is provided in the Dose Escalation Management Plan.

3.3. Phase 2 – Dose-Expansion in Relapsed/Recurrent GBM

Dose-expansion components will 1) assess safety, PK/PD and efficacy of USL311 as a single agent in subjects with relapsed/recurrent GBM eligible for re-resection (Part 3), and 2) assess safety, PK/PD and efficacy of the combination of USL311 with lomustine in subjects with relapsed/recurrent GBM eligible for re-resection (Part 4).

The dose-expansion component will determine the PFS-6m of single-agent USL311 (Part 3) and of USL311 in combination with lomustine (Part 4).

Figure 2: Study Schematic for Phase 2



3.3.1. Part 3 – Dose-Expansion in GBM of Single Agent USL311

Once the RP2D of single agent USL311 has been determined by the DEC and Sponsor, Part 3 will assess preliminary efficacy of single agent USL311. Subjects in Part 3 will participate in the following visits:

- Screening Visit (within 28 days of Pre-surgical Dosing, Day 1 for those randomized to pre-surgical treatment and within 28 days of Surgery for those randomized to no pre-surgical treatment)
- Pre-surgical Dosing Phase Visits (Visits on Day 1, Day 2, Day 3, Day 5, and Day 8+ only for those randomized to pre-surgical treatment)
- Surgery (after one to three weeks of treatment during Pre-surgical Dosing for those randomized to pre-surgical treatment; following screening for those randomized to no pre-surgical treatment)
- Post-surgical Treatment Phase Visits for Cycle 1 (Visits on Day 1, Day 2, Day 8, and Day 15)
- Post-surgical Treatment Phase Visits for Cycle 2+ (Visits on Day 1, Day 8, and Day 15)
- End of Treatment Visit (within 14 days of decision to discontinue on-study treatment)
- Follow-up Visit (within 28 days of last dose on USL311; may be same visit as End of Treatment Visit)
- Long Term Follow-up Visits (quarterly visit or phone call until subject death or study termination)

Approximately 20 subjects with relapsed/recurrent GBM, who are candidates for re-resection, will be enrolled. Subjects will be randomized to receive either once daily oral pre-surgical treatment with USL311 or no USL311 treatment prior to surgery (see Section 6.2). Subjects randomized to receive pre-surgical treatment with USL311 are required to receive at least one week of treatment prior to surgery, and may receive up to a maximum of three weeks of treatement prior to surgery at the single agent USL311 RP2D defined in Part 1. The length of treatment will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Following surgery, all subjects (including those randomized to no pre-surgical treatment) will initiate treatment with once daily, oral USL311 within 28 days post-surgery, although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy. USL311 will be administered once daily during every 3-week (21-day) Cycle with no breaks between cycles. Subjects will be monitored by study staff for at least 4 hours after administration of their first dose of USL311. Treatment Cycles will be repeated every 3 weeks (21 days) until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study.

Exploratory (translational) studies will be performed on resected tissue following surgery to determine biodistribution of USL311 in the tumor and to assess PD markers (see Section 8.7.2).

3.3.2. Part 4 – Dose Expansion in GBM of USL311 in Combination with Lomustine

Once RP2D of USL311 in combination with lomustine has been determined by the DEC and Sponsor, Part 4 will assess preliminary efficacy of the combination. Subjects in Part 4 will participate in the following visits:

- Screening Visit (within 28 days of Pre-surgical Treatment Dosing, Day 1 for those randomized to pre-surgical treatment and within 28 da ys of Surgery for those randomized to no pre-surgical treatment)
- Pre-surgical Dosing Phase Visits (Visits on Day 1, Day 2, Day 3, Day 5, and Day 8+; only for those randomized to pre-surgical treatment)
- Surgery (between ≥ 24 hours and < 8 days after the last dose administered during Pre-surgical Dosing for those randomized to pre-surgical treatment; following screening for those randomized to no pre-surgical treatment)
- Post-surgical Treatment Phase Visits for Cycle 1 (Visits on Day 1, Day 2, Day 8, Day 15, Day 22, Day 29, and Day 36)
- Post-surgical Treatment Phase Visits for Cycle 2+ (Visits on Day 1, Day 2, Day 8, Day 15, Day, 22, Day 29, and Day 36)
- End of Treatment Visit (within 14 d ays of decision to discontinue on-study treatment)
- Follow-up Visit (within 28 days of last dose on USL311; may be same visit as End of Treatment Visit)
- Long Term Follow-up Visits (quarterly visit or phone call until subject death or study termination)

Approximately 20 subjects with relapsed/recurrent GBM, and who are candidates for re-resection, will be enrolled. Subjects will be randomized to receive once daily oral pre-surgical treatment with USL311 or no USL311 treatment prior to surgery (see Section 6.2). Subjects randomized to receive pre-surgical treatment are required to receive at least one week of treatment of USL311 (at the RP2D defined in Part 2) prior to surgery, and may receive up to a maximum of three weeks of treatment prior to surgery at the USL311 RP2D defined in Part 2. The length of treatment received will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Following surgery, all subjects (including those randomized to no pre-surgical treatment) will initiate treatment with once daily, oral USL311 in combination with lomustine within 28 days post-surgery, although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy. USL311 will be administered daily during every 6-week (42-day) cycle, with no breaks between cycles. Lomustine will be

administered on Day 2 of every 6-week (42-day) cycle. Subjects will be monitored by study staff for at least 4 hours after administration of their first dose of USL311. Treatment cycles will be repeated every 42 days until disease progression, unacceptable toxicity, withdrawal of consent, Investigator decision to discontinue treatment, or Sponsor decision to terminate the study.

Exploratory (translational) studies will be performed on resected tissue following surgery to determine biodistribution of USL311 in the tumor and to assess PD markers (see Section 8.7.2).

3.3.3. Surgical Re-Resection

During Part 3 and Part 4, subjects with relapsed/recurrent GBM will have a surgical re-resection of the tumor performed as part of their standard-of-care treatment per the Investigator's discretion. Subjects will complete the surgery following the investigational site's surgical procedures. During surgery, a CSF sample (see Section 8.6.2) and tumor tissue sample (see Section 8.7.2) will be collected and stored according to instructions in the lab manual. Participation in Part 3 and Part 4 will not influence the area of surgical re-resection or the amount of tumor resected, as these will be determined by the Investigator as a component of their standard-of-care treatment.

The subject will be informed in the consent form about the reason for collecting samples and how the samples will be obtained and stored. Any data obtained from analysis of tumor tissue or CSF samples will be used for translational research, and subject confidentiality will be preserved internally and in any presentations or publications.

A baseline response assessment using the RANO criteria will be performed ≤ 72 hours after surgery. If initiation of treatment following surgery is delayed for 3-4 weeks, a repeat baseline response assessment using the RANO criteria will be performed ≤ 7 days prior to study drug(s) dosing. Following surgery, all subjects will subsequently receive single agent USL311 (Part 3) or USL311 in combination with lomustine (Part 4) within 28 days (4 weeks), although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy.

3.4. End of Study

The definition for the end of the study is the time of data cutoff for the final analysis or the time of last subject/last visit, whichever occurs later. The end of the study will be reached after all subjects have received 6 months of treatment or have discontinued.

4. RATIONALE

4.1. Rationale for Study

The improvement of treatment for relapsed/recurrent GBM remains a significant unmet medical need as there are currently limited options for patients with relapsed/recurrent GBM. One potential target for treatment of GBM, as well as other solid tumor types, is the chemokine receptor, CXCR4. CXCR4 is overexpressed in a wide range of solid tumors, including GBM, and overexpression appears to be a negative prognostic factor for overall survival (27, 28). Examples of these tumor types in which CXCR4 overexpression is correlated with a poor prognosis include, but are not limited to, GBM (29), renal cell carcinoma (30), hepatocellular carcinoma (31), colorectal cancer (32), non-small cell lung cancer (33, 34), breast cancer (35), esophageal cancer (36) and prostate cancer (37).

Additionally, several studies have demonstrated the existence of CSCs for a variety of different tumor types which constitute a reservoir of self-sustaining tumor cells with the ability to maintain tumor growth. Many of these cells express CXCR4 and respond to SDF-1, suggesting that CSCs represent a subpopulation capable of initiating metastasis in response to CXCR4 activation (24). As the CXCR4 receptor and its chemokine ligand SDF-1 have roles in tumor growth and metastasis, antagonism of the SDF-1/CXCR4 signaling pathway is expected to inhibit migration and self-renewal of CSCs while also rendering these cells more sensitive to concurrent therapies.

These data from the literature provide the rationale for investigating USL311 as a potential anticancer agent for the treatment of solid tumors, including GBM.

4.2. Rationale for Study Design

Dose-escalation using a mCRM design in subjects with advanced solid tumors is a standard method in the early phase clinical development of novel anticancer treatment. Subjects with advanced solid tumors are those for which no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment will participate in the dose escalation portion of the study. Dose escalation will allow for the identification of the MTD and RP2D of single agent USL311 (Part 1) and the identification of the MTD and RP2D of USL311 in combination with lomustine.

Dose expansion will allow a more robust investigation of the preliminary efficacy as well as the safety, PK and PD of study drug USL311 alone, and in combination with lomustine, in the target population of relapsed/recurrent GBM.

Surgical re-resection is performed as part of standard-of-care for select GBM patients. The pre-surgical study design was employed in a recent study in which patients with GBM requiring tumor re-resection for recurrent disease received preoperative lapatinib (48). Lapatinib concentrations and epidermal growth factor receptor (EGFR) phosphorylation were then assessed in surgical specimens. A similar study design was used to evaluate the efficacy, PK and PD of effects of cilengitide (49). In this study, subjects were initially randomized to cilengitide treatment or no treatment for the pre-surgical period of the study, and all subjects continued treatment post-surgery until progressive disease. The study design involving

surgical re-resection as part of the evaluation of USL311 allows for the evaluation of both PK and PD markers in the targeted brain tissue.

Additionally, surgery at progression does not appear to be a prognostic marker for improved PFS-6m or OS for patients with relapsed/recurrent GBM (50); thus, PFS-6m results reported in this study would depend on effects from study drug(s) administration and not from the surgical procedure. Furthermore, USL311 does not affect wound healing in animal models; therefore, patients who have recently undergone surgery should not exhibit delayed healing.

4.3. Rationale of Study Population

Subjects with advanced solid tumors for which no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment, will be selected to participate in the Phase 1 dose-escalation portion of this study. This population commonly participates in Phase 1 dose-escalation oncology studies to determine the RP2D. Subjects with advanced solid tumors commonly participate in studies with single agent study drug and in studies with a combination of agents for both study drug and currently approved agents. Due to the mechanism of action for USL311 it is possible many different types of solid tumors may experience therapeutic benefit from treatment.

Adult subjects with relapsed/recurrent GBM eligible for re-resection will participate in the Phase 2 dose-expansion portion of this study. Relapsed/recurrent GBM subjects are the target population for single agent USL311 and USL311 in combination with lomustine. Due to the mechanism of action and CNS penetration of USL311, GBM patients are expected to experience therapeutic benefit as described in Section 4.4.

4.4. Rationale for Study Treatment

The study drug is USL311, a CXCR4 antagonist under investigation for the treatment of relapsed/recurrent GBM. Evidence from the literature suggests possible efficacy of CXCR4 antagonists for the treatment of GBM. Additionally, evidence from the non-clinical studies suggest possible efficacy of USL311 for the treatment of GBM. Rationale for the recommended starting dose and administration is provided in Section 6.1.2. USL311 is a CXCR4 antagonist that has demonstrated nonclinical efficacy in both in vitro and in vivo models when administered in combination with cytotoxics, anti-angiogenics and/or radiation. Specifically, in the murine models, single agent USL311 has demonstrated CXCR4 blockade which resulted in delayed progression of xenograft tumors derived from GBM-based cell lines, and has demonstrated the ability to delay tumor regrowth in combination with chemotherapy and radiotherapy. Notably, USL311 demonstrates extensive uptake in a range of tissues and crosses the blood-brain barrier, making it suitable for the treatment of CNS malignancies, including GBM. The extensive tissue uptake is attributed in part to “lysosomal trapping” of USL311, which may also contribute to USL311’s putative anti-cancer effect through inhibition of autophagy, a homeostatic cellular recycling process which is disrupted by other lysosomotropic drugs leading to enhanced tumor cell death (see Section 1.2.3.1).

Lomustine is the selected combination agent for the clinical development of USL311. Lomustine, a cytotoxic drug, is a nitrosourea used in the treatment of certain oncologic conditions and functions as an alkylating agent. Although lomustine is primarily used in brain

tumors (both primary and metastatic) and Hodgkin's disease, it has also been used or under investigation for use in the second or third line setting, in combination with other therapeutics, in a number of other cancers including; lung cancer, multiple melanoma, colorectal cancer, liver cancer and advanced breast cancer. Lomustine is commonly used in recurrent GBM and has also been used as the comparator in recent registration-directed trials in this setting. Lomustine is an acceptable choice of chemotherapy to combine with USL311 given that it has shown improved median and 6-month PFS as monotherapy for relapsed/recurrent GBM compared to historical controls and has been combined with other novel agents in relapsed/recurrent GBM (20, 21).

5. SUBJECT POPULATION

During Phase 1 dose-escalation, the study population will be subjects with advanced solid tumors for which no standard-of-care treatment is recognized or who have failed or are intolerant to the standard-of-care treatment.

During Phase 2 dose-expansion the study population will be subjects with relapsed/recurrent GBM whom are candidates for re-resection, having previously received definitive first line treatment.

5.1. Number of Subjects

The total number of subjects will depend on the number of dose levels assessed in Phase 1 (Parts 1a/1b and 2). It is expected that approximately 6-40 subjects with advanced solid tumors be enrolled for Part 1b single agent USL311 dose-escalation and approximately an additional 6-40 subjects with advanced solid tumors be enrolled for Part 2 USL311 in combination with lomustine dose-escalation. It is expected that approximately 20 subjects with relapsed/recurrent GBM will be enrolled for each Phase 2 (Parts 3 and 4) dose-expansion cohort, with a total of approximately 40 subjects. A total of 13 subjects were enrolled in Part 1a with IV USL311. The total number of subjects for Phase 1 and 2 combined is expected to be approximately 65-133 subjects.

5.2. Eligibility Criteria

Questions about eligibility criteria should be addressed prior to enrollment. The eligibility criteria for this study have been carefully considered. Eligibility criteria are standards used to ensure that subjects who enter this study are medically appropriate candidates for this therapy. For the safety of the subjects, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, there will be no exceptions to eligibility requirements at screening.

5.2.1. Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

Criteria for both Phase 1 dose-escalation and Phase 2 dose-expansion:

1. Provide signed and dated informed consent prior to study-specific screening procedures
2. ≥ 18 years old
3. Karnofsky performance status (KPS) ≥ 70
4. Must have adequate bone marrow and renal/hepatic function at the Screening Visit and at Baseline, defined as:
    a. ANC ≥ 1,500/mm^3 without granulocyte colony-stimulating factor (G-CSF) support within 7 days preceding the lab assessment
    b. Platelet count ≥ 100,000/mm^3, without transfusion within 7 days preceding the lab assessment
    c. Hgb ≥ 9 g/dL, without transfusion support within 7 days preceding the lab assessment

d. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≤ 3 times upper limit of normal (ULN)
e. Total serum bilirubin ≤ 1.5 times ULN, except in subjects with known Gilbert's Syndrome ≤ 3 times ULN
f. Serum creatinine ≤ 1.5 times ULN with an estimated creatinine clearance of ≥ 60 mL/min (calculated by the Cockcroft-Gault equation), or creatinine clearance corrected for body surface area (BSA) ≥ 60 mL/min/1.73 m^2 (determined from 24-hour urine collection) per Investigator discretion
g. Activated partial thromboplastin time/partial thromboplastin time (aPTT/PTT) and prothrombin time (PT) ≤ 1.5 ULN
h. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels within normal limits, or above the ULN if considered not clinically significant per the Investigator. Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below lower limit of normal must be corrected to within the normal range, or above the ULN if considered not clinically significant per the Investigator, by supplementation prior to starting study drug(s).

5. Disease-free period of > 2 years from any other previous malignancies, excluding curatively treated basal cell carcinoma, squamous cell carcinoma of the skin, or carcinoma *in situ* of the cervix. Subjects with prostate cancer Stage 1 that do not require treatment may also be included.

6. Women of childbearing potential (WOCBP) must have two negative pregnancy tests, the first during Screening and the second within 24 hours prior to first administration of study drug(s) and must agree to use highly effective physician-approved contraception (see Appendix 2) from Screening to 90 days following the last study drug administration. Male subjects must be surgically sterile or must agree to use highly effective physician-approved contraception from Screening to 90 days following the last study drug administration (a barrier method of contraception must be employed by all subjects [male and female], when having sexual intercourse, regardless of other methods)

    a. Females are considered not of childbearing potential if they meet any of the following criteria:

        - Postmenopausal with > 1 year since last menses and:
            - If younger than 65 years old, with a FSH > 40 mIU/mL
            - If ≥ 65 years old and not on HRT, with a FSH > 30 mIU/mL
            - If ≥ 65 years old and on HRT, the FSH requirement in not applicable. Postmenopausal females on HRT will be allowed if the treatment is stable for at least 6 months prior to dosing of study drug(s)
        - Written medical documentation of being sterilized (e.g. hysterectomy, double oophorectomy, bilateral salpingectomy). Note: Tubal ligation is not considered a form of permanent sterilization.

7. Must be able and willing to comply with the study visit schedule and study procedures

8. Must be able to take oral medications

9. Must have available archived tumor tissue and be willing and able to provide consent for study access to such tissue
10. For subjects with a history of seizures, must be adequately controlled on a stable regimen of anti-epileptic drugs

Criteria for Phase 1 only, dose-escalation in advanced solid tumors:

11. Histologically or cytologically documented diagnosis of solid tumor for which no standard therapy is recognized or have failed or intolerant to the standard-of-care treatment
12. Inoperable metastatic or locally advanced, unresectable disease
13. Subjects must have either evaluable or measurable disease
14. Subjects with treated (surgically excised or irradiated) and stable brain metastases are eligible as long as the subject has adequately recovered from treatment and the treatment was ≥ 28 days prior to initiation of study drug(s) and baseline brain computed tomography (CT) with contrast or magnetic resonance imaging (MRI) ≤ 14 days of initiation of study drug is negative for new brain metastases.

Criteria for Phase 2 only, dose-expansion in relapsed/recurrent GBM:

15. Histologically confirmed diagnosis of GBM
16. Subjects must have documented recurrence after first-line treatment
17. Prior first-line treatment must have included radiation and temozolomide
18. Subject is suitable for re-resection, per Investigator discretion, as a component of their clinical care
19. No more than one prior resection (Note: biopsy does not count as prior resection)

5.2.2. Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subjects who have had recent systemic anticancer therapies, interventional device treatment and/or radiotherapy either within 14 days prior to first dose of study drug(s) or have not recovered (to grade ≤ 1) from all clinically significant toxicities related to prior therapies
2. Subjects who have had any major surgery (not including re-resection surgery required in Phase 2) within 28 days prior to first dose of study drug(s), or minor surgery within 14 days prior to first day of study drug(s)
3. For 14 days prior to first day of study drug(s) treatment, administration of any strong cytochrome P450 3A4 (CYP3A4) inducers including, but not limited to, the following: carbamazepine, ethotoin, mephenytoin, phenobarbital, phenytoin, primidone, rifabutin, rifampin, and St. John's Wort

4. For 14 days prior to the first day of study drug(s) treatment, administration of any strong cytochrome P450 3A4 (CYP3A4) inhibitors including, but not limited to, the following: amprenavir, ataznavir, boceprevir, clarithromycin, conivaptan, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, nefazodone, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, and voriconazole
5. For 14 days prior to first dose of study drug(s) treatment, administration of any agent with moderate-to-high risk to prolong the QTc interval or to cause Torsades de Pointes (see Appendix 3)
6. Subjects who have been treated with an investigational agent or investigational interventional device within 21 days prior to the first dose of study drug(s)
7. Subject is growth factor dependent or transfusion dependent, or has received growth factor support or transfusion support within 14 days prior to the first dose of study drug(s)
8. History of significant cardiac disease. Significant cardiac disease includes the following:
    a. Second/third degree heart block
    b. Significant ischemic heart (e.g. myocardial infarction, unstable angina, Grade 3 or 4 [Canadian Cardiovascular Society] angina, hospitalization for ischemic heart disease) within 2 years of first dose of study drug(s)
    c. Family history of long QT syndrome; mean Fridericia corrected QT interval (QTcF) > 450 msec on at least two separate ECGs prior to study start
    d. Poorly controlled hypertension per Investigator opinion
    e. Congestive heart failure of NYHA Class II or worse (slight limitation of physical activity; comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea)
9. Episode of status epilepticus within 1 year prior to the first dose of study drug(s)
10. Pregnant or breastfeeding
11. Any other significant co-morbid conditions that in the opinion of the Investigator would impair study participation or cooperation, including known or suspected history of significant allergic reaction or hypersensitivity to any components of the USL311 formulation

Criteria for Phase 1 only, dose-escalation in advanced solid tumors

12. Subjects with lymphoma as primary cancer

Criteria for Phase 2 only, dose expansion in relapsed/recurrent GBM:

13. Subjects unable or unwilling to consent to the provision of resected tissue after surgery
14. Prior treatment with plerixafor or another CXCR4 inhibitor
15. Prior treatment with bevacizumab
16. Prior treatment with lomustine and/or carmustine

Criteria for all cohorts receiving oral USL311:

17. Participants with any active medical condition or previous major abdominal surgery or procedure that might, in the investigator's opinion, have a significant effect on USL311 absorption

5.3. Prior and Concomitant Medications

All prescription and over-the-counter medications taken by a subject from 28 days prior to the first dose of study drug(s) until the Follow-up Visit will be recorded on the designated electronic case report form (eCRF). The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any required medications may be prescribed without prior approval, but the Medical Monitor or designee must be notified of the use of any prohibited medications immediately thereafter. Any concomitant medications changed, added or discontinued during the study should be recorded on the eCRF, including the indication and the start and end dates of treatment, as applicable.

5.3.1. Permitted Medications

All supportive therapy for optimal medical care will be given during the study period at the discretion of the Investigator(s) within the parameters of the protocol and documented as concomitant medications on the designated eCRF. The following medications are permitted, per Investigator discretion, but may have limitations in place during the duration of the study.

- Anticonvulsants: Anticonvulsants may be used as clinically indicated. Doses at study entry and at specific time points of the treatment must be recorded. Note: The use of enzyme inducing antiepileptic drugs (EIAEDs) are not permitted.
- Antiemetics: Use of prophylactic oral antiemetics should be used consistent with ASCO guidelines (51) or other institutional practice guidelines. Use of antiemetics with known QTc prolongation potential are not permitted during the study. Only the antiemetic agents listed below are permitted:
  - Granisetron, palonosetron, aprepitant, fosaprepitant, dexamethasone, and lorazepam
- Anticoagulants: Prophylactic therapy with aspirin, a low-molecular weight heparin, or a Factor Xa inhibitor is acceptable. Warfarin and dabigatran are not permitted.
- Corticosteroids: Corticosteroids may be used as clinically indicated. Doses at study entry and at specific time points of the treatment must be recorded. **Note:** Documentation of corticosteroid use is important for evaluation of response using the RANO criteria (see Appendix 6).
- Electrolyte Replacement Therapy: Electrolyte replacement administered should stabilize the subject within the normal range, or above the ULN if considered not clinically significant per the Investigator, prior to dosing of study drug(s).
- Hematopoietic Growth Factors: Erythrocyte growth factors (e.g. erythropoietin) are not to be administered prophylactically but may be prescribed per institutional guidelines for anemia if this is deemed appropriate by the Investigator. Transfusions are also permitted. Granulocyte growth factors (e.g., G-CSF and GM-CSF) are not to be administered prophylactically but may be prescribed by the Investigator for severe neutropenia if this is deemed appropriate. However, study drug(s) should be held for at least 24 hours after the expected duration of activity for any granulocyte

growth factors. The administration of granulocyte growth factors should be consistent with ASCO guidelines (52).

- Systemic Anesthetics: Systemic anesthetics are permitted but should not be given within 24 hours of USL311. USL311 may decrease the effectiveness (i.e., higher doses of the anesthetic may be required) of systemic anesthetics when used within 24 hours of the infusion. The Medical Monitor should be informed if systemic anesthetics are to be administered outside of the surgical re-resection required for Phase 2.
- Low Risk QTc Interval Prolonging Agents: Medications with low risk to prolong the QTc interval may be used at the discretion of the Investigator.

5.3.2. Prohibited Medications

The following medications are prohibited during the duration of the study:

- Other Anticancer Agents: Subjects may not receive any other anticancer therapies while on study prior to disease progression. This includes systemic anticancer (both cytotoxic and non-cytotoxic) therapies, interventional devices, and radiotherapy.
- CYP3A4 Strong Inhibitors and Inducers: Subjects may not receive any substances which are known strong inducers or inhibitors of CYP3A4 (see Table 17), including drugs, herbs, and nutritional supplements, as they may alter the PK of USL311. If the subject is taking any of these restricted substances at the time of screening, the time between the last dose of that substance and study drug(s) administration must be at least 14 days.

Table 17: Prohibited Cytochrome P450 Enzyme Interaction Medications

Cytochrome P450 Enzyme	Medication Name
Strong CYP3A4 Inducers	carbamazepine, ethotoin, mephenytoin, phenobarbital, phenytoin, primidone, rifabutin, rifampin, and St. John's Wort
Strong CYP3A4 Inhibitors	amprenavir, ataznavir, boceprevir, clarithromycin, conivaptan, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, nefazodone, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, and voriconazole

- Moderate-to-High Risk QTc Interval Prolonging Agents: Subjects may not receive any medication with a known moderate-to-high probability of prolonging the QTc interval or to cause Torsades de Pointes (see Appendix 3). If the subject is taking any of the restricted medications at the time of screening, the time between the last dose of the medication and study drug(s) administration must be at least 14 days. If one of these medications needs to be administered during the study (i.e., an antibiotic with moderate-to-high QTc interval prolongation risk), the Medical Monitor must be contacted before initiating treatment with the restricted medication and attempts

should be made to avoid administration within 24 hours after the administration of USL311.

- Gastric acid lowering agents – For subjects receiving oral USL311 only: subjects should try to limit ta king antacids within 1 hour prior to or after USL311 administration. If a patient is taking a histamine type 2 antagonist or a proton pump inhibitor, it is preferable the subject take the medication at least 1 hour after USL311 administration.
- Other Investigational Agent: No other investigational agent (medication or device) may be used during the duration of the study.

5.4. Dietary and Other Protocol Restrictions

There are dietaryrestrictions during the course of this study.

For subjects receiving oral USL311 only:

In Parts 1b, 2, 3 and 4, USL311 will be administered as oral tablets once daily in the morning under fasted conditions for 2 hours before and 2 hours after the dose on PK sampling days. For all other outpatient dosing days the subject should be instructed to make an effort to take the oral tablets at least 1 hour before or at least 1 hour after their meal, and they will be instructed to document in their diary the time of any food eaten prior to dosing and for 1 hour after dosing.

5.5. Subject Withdrawal or Discontinuation

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment.

The Sponsor must be notified of all subject withdrawals as soon as possible. The Sponsor also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or Sponsor may withdraw administration of study drug include, but are not limited to, the following:

- Subject experiences a serious or intolerable AE or clinically significant laboratory abnormality
- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up
- Subject enrolls in a different clinical study

- Subject becomes pregnant (see Section 9.3.3 for details on the reporting procedures to follow in the event of pregnancy)
- Subjects may be removed from study if considered by the Investigator or Sponsor to be in the best interest of the subject

For subjects who withdraw, procedures and assessments for the End of Treatment Visit (see Section 7.3) should be performed (unless the subject withdraws consent to do so). If the subject withdraws consent, this request must be documented in the study records, and signed by the Investigator. At this time, the subject will be asked if he/she agrees to be followed-up for collection of survival information. This agreement must be documented in the study records and signed by the Investigator. The study staff may also use a public information source (e.g., county records) to obtain information about survival status.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. Additionally, a documented effort must be made to request for follow-up survival information. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for the evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

5.6. Subject Identification and Replacement of Subjects

Subjects in Phase 1 (Parts 1 and 2 dose-escalation) who discontinue the study before they have completed the first cycle of treatment for reasons other than toxicity will be replaced. A subject must meet one of the following criteria to be evaluable for cohort DLT/MTD/dose-escalation decisions:

- Received all 3 scheduled infusions or received ≥ 16 of 21 daily oral doses of single agent USL311 during Cycle 1, has completed Cycle 1-Day 21 and has not experienced a DLT (Part 1)
- Received all of the 6 scheduled infusions or received ≥ 32 of 42 daily oral doses of USL311 as well as the Cycle 1 lomustine dose, has completed Cycle 1-Day 49 and has not experienced a DLT (Part 2)
- Experienced a DLT
- Withdrew from the study prior to completing Cycle 1 due to an AE considered by the investigator to be at least possibly related to study drug(s)

If a subject does not meet any of these criteria, the subject is not evaluable for dose-escalation decisions and will be replaced.

In Phase 2, all subjects will be treated until progression or another withdrawal criterion is met (see Section 5.5). Subjects will be enrolled to have approximately 20 evaluable subjects in Part 3 and 20 evaluable subjects in Part 4.

5.7. Duration of Subject Participation

The duration of each subject's participation will be variable. Subjects who tolerate Cycle 1 treatment and do not have progressive disease, treatment failure or relapse are eligible to continue to receive treatment until they experience unacceptable toxicity, disease progression, meet any of the withdrawal criteria, or the study is terminated by the Sponsor. Subjects removed from the study for unacceptable AEs will be followed until AE resolution (return to normal or baseline) or the subject's condition has stabilized to the satisfaction of the Investigator. Additionally, subjects will be followed quarterly (every 3 months ± 1 week) according to the criteria defined in Section 7.4.

5.8. Informed Consent

Each prospective subject will provide written informed consent before any screening evaluations or other study procedures are performed.

Informed consent will be given by means of a standard statement, written in non-technical language, which explains the nature of the study, its purpose, procedures, expected duration, alternative therapy available, the benefits and risks involved in study participation, and any discomfort study participation may entail. The Investigator or his/her designee must emphasize to the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled or affecting subsequent medical treatment or relationship with the treating physician.

The subject will read and consider the statement and be allowed to ask any questions before signing and dating it, and he/she should be given a copy of the signed and dated document. The person conducting the informed consent discussions must personally date and sign the ICF. The Investigator will retain the original signed ICF.

Some subjects will provide written consent in the form of an assent form before any screening evaluation or other study procedure is performed, if required by local law or IRB/IEC policy. The assent form will provide similar information as the informed consent form, and the same procedures will be followed as described above for the informed consent form. The assent form must be signed and dated by the subject and the qualified research professional obtaining the assent.

No subject can enter the study and no study-specific procedures can be performed before informed consent has been obtained.

Prior to consenting subjects, the Investigator or designee must submit the informed consent form with the study protocol for IRB/IEC approval. All proposed informed consent forms must be reviewed and approved by the Sponsor or its designee before submission to the IRB/IEC.

All informed consent and assent forms will be reviewed by the IRB/IEC and approved (IRB) or a favorable opinion received (IEC) before use in this study. Informed consent will be obtained in a manner consistent with Good Clinical Practice (GCP)/International Conference on Harmonization (ICH). A copy of the approved version must be provided to the Sponsor or the study monitor after IRB approval/IEC favorable opinion.

5.9. Authorization to Use and Disclose Medical Information

Each subject will be identified by initials (3 letters) and a unique subject number. In countries where the subjects' initials cannot be used by local regulations, study centers will use dummy initials.

All countries must follow local law(s) for authorization to use and disclose medical information.

Information obtained from standard-of-care procedures, including those performed prior to the subject signing the informed consent, that occur within the protocol-defined windows (e.g., Screening window) may be obtained and utilized when applicable.

The remainder of this section only applies to study centers in the United States.

Under US federal law, subject study records cannot be used or disclosed for research purposes unless an authorization to use and disclose medical information is signed by each subject prior to participation in the study. The Investigator or designated assistant will explain to each subject the purpose of the subject authorization and the disclosures agreed to by signing the authorization document. Subjects will be given an authorization document and will have the opportunity to ask questions. Subjects must also be informed of the following:

- They may not participate in the study unless the authorization is signed; however, they have the right to revoke this authorization (in writing) at any time
- If they discontinue from the study, they are not required to revoke the authorization to use and disclose their medical information
- If they discontinue from the study and do decide to revoke their authorization to use and disclose their medical information, the information that has already been collected in their study records may be used and disclosed as necessary to protect the integrity of the research project

After this explanation and before any study-specific procedures have been performed, the subject will voluntarily sign and date an authorization document. Prior to participation in the study, the subject will receive a copy of the signed and dated written authorization.

Authorization to disclose Protected Health Information for research will be obtained in accordance with HIPPA regulations 45 CFR Parts 160 and 164.

6. STUDY TREATMENT INFORMATION

6.1. Study Treatment Administration and Doses

The study drug used for the IV portion of Part 1 (Part 1a) is USL311 Injection Solution. The study drug used for the oral portion of Part 1 (Part 1b) as well as for Parts 2-4, is USL311 20 mg or 100 mg tablets.

The combination agent used in this study is lomustine (CCNU).

6.1.1. Directions for Administration

During Part 1 and Part 3, USL311 will be administered as single agent and during Part 2 and Part 4, USL311 will be administered in combination with lomustine.

6.1.1.1. USL311 IV Preparation

USL311 Injection Solution requires dilution with a 250 mL infusion bag of 0.9% Sodium Chloride Injection, USP prior to administration to produce a final concentration of the intended dose.

USL311 IV is dosed based on the subject's BSA calculated using the DuBois and DuBois equation.

First, aseptically withdraw the same volume required for the dose of USL311 Injection Solution from the 250 mL infusion bag of 0.9% Sodium Chloride and dispose of the waste. The removal of this volume will ensure the final volume of the admixture will be 250 mL. Next, aseptically withdraw the volume of USL311 from the product vial needed for the required dose of USL311 and immediately transfer to the 250 mL infusion bag of 0.9% Sodium Chloride. After transferring, thoroughly mix the contents of the infusion bag. The admixture should be a clear and colorless to slightly yellow solution.

Use only 0.9% Sodium Chloride Injection, USP, for dilution, as outlined above. No other diluents have been assessed for compatibility.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Vials of USL311 Injection Solution are for single use only. Any unused solution should be discarded according to institutional procedures.

6.1.1.2. USL311 Admixture Stability

USL311 Injection Solution contains no antimicrobial preservative. The admixture should be prepared as close as possible to the time of subject administration. Once diluted, the final admixed product may be stored for no more than **6** hours at room temperature (15-30°C or 59-86°F) or no more than 24 hours under refrigeration (2-8°C or 36-46°F). Administration of the admixed product must be complete within 24 hours.

6.1.1.3. USL311 IV Administration

USL311 will be administered through a peripheral IV line over a nominal duration of 120 minutes (2 hours, Protocol Amendment #2) or 240 minutes (4 hours, Protocol Amendment #3)

once weekly, although longer infusion durations may be considered as warranted based on clinical observations during the study.

If the subject has other forms of venous access (peripherally inserted central catheter line [PICC line] or central line), these may be used instead of a peripheral IV line, per Investigator discretion. USL311 start and stop times of the infusion will be documented, and the stop time should be within +/- 10 minutes of the nominal time relative to the start of infusion (i.e., start time plus 240 minutes). Any unplanned interruption in USL311 infusion needs to be documented along with the corresponding start and stop times of the infusion. USL311 will be administered on Days 1, 8, and 15 of a 21-day (3 week) cycle in Part 1a.

6.1.1.4. USL311 Oral Administration

In Parts 1b, 2, 3 and 4, USL311 will be administered as oral tablets once daily in the morning under fasted conditions for 2 hours before and 2 hours after the dose on PK sampling days. For all other outpatient dosing days, the subject should be instructed to make an effort to take the oral tablets at least 1 hour before or at least 1 hour after their meal, and they will be instructed to document in their diary the time of any food eaten prior to dosing and for 1 hour after dosing.

If a dose is missed in the morning, and fewer than 12 hours have elapsed since the planned administration time, the subject should take the missed dose. If more than 12 hours have elapsed since the planned administration time, the missed dose should be skipped. The time and date of daily administration of USL311 will be documented in a diary provided to the subject, see Section 6.6.1.

6.1.1.5. Lomustine Administration

Lomustine will not be administered in Part 1 and Part 3 or during the pre-surgical treatment period of Part 4.

Lomustine should be administered per the instructions found in the package insert (see Appendix 4). It is recommended to administer lomustine on an empty stomach to help prevent lomustine induced nausea and vomiting. Lomustine is dosed based on the subject's BSA, calculated using the DuBois and DuBois equation. All doses of lomustine should be rounded to the nearest 10 mg by the Investigator.

In Part 2, Cycle 1 only, a single oral dose of lomustine will be administered on Day 1 as part of a 49-day (7-week) cycle and will be administered in the clinic. For all subsequent cycles in Part 2 (Cycle 2+), a single oral dose of lomustine will be administered on Day 2 as part of a 42-day (6-week) cycle and will be administered in the clinic during Cycle 2 but for all subsequent cycles, lomustine may be dispensed for outpatient administration as allowed per site standard of practice. If outpatient dosing of lomustine is not allowed per site standard of practice, lomustine will be administered in the clinic.

In Part 4, dosing with lomustine will initiate following surgical re-resection and will be administered as a single oral dose on Day 2 as part of a 42-day (6-week) cycle. The first dose of lomustine (Cycle 1) will be administered in the clinic, whereas for all subsequent cycles (Cycle 2+), lomustine may be dispensed for outpatient administration. If outpatient dosing of

lomustine is not allowed per site standard of practice, lomustine will be administered in the clinic.

For all outpatient doses (if applicable) in Parts 2 and 4, the subject will be instructed to take lomustine as a single dose on Cycle Day 2. If lomustine is administered as an outpatient dose, clinic staff will follow-up by phone with the subject within 48 hours after Day 2 to collect the following: time and date of lomustine administration, concomitant medications, and AEs.

Note: In the case of emesis after dosing with lomustine and the expulsion of the capsule, lomustine will not be re-dosed.

6.1.2. Selection of Treatment and Doses

6.1.2.1. Phase 1

Part 1a – Single Agent IV USL311 Dose Escalation:

The first-in-human starting dose of USL311 will be 60 mg/m2 administered as a 2-hour IV infusion. The Severely Toxic Dose in 10% of animals (STD 10) was determined as 100 mg/kg USL311 administered as a weekly 2-hour IV infusion in the rat. Based on scaling by BSA, the human equivalent dose to the STD 10 is 600 mg/m2 and thus the starting dose is equivalent to 1/10th of the rat STD 10. This dose is lower than the projected first-in-human starting dose of 267 mg/m2 calculated as 1/6 of the Highest Non-Severely Toxic Dose (HNSTD) in dogs (80 mg/kg as a weekly 2-hr infusion).

A weekly dosing schedule was selected as USL311 exhibits a prolonged terminal half-life following IV administration in nonclinical species. This is attributed to the extensive tissue uptake and slow elimination from the tissues including the brain. The predicted terminal half-life in humans is estimated on the order of days to weeks and thus target USL311 concentrations may be achieved and maintained with a weekly IV dosing schedule.

As of November 17, 2016, data from 8 subjects participating in Part 1 of this study (as defined prior to Protocol Amendment #3) has been collected at USL311 doses of 60, 120 and 180 mg/m2 via 2 hr IV infusion. Interim data from these subjects suggested a potential USL311-related increase in the the QT interval, with an apparent correlation with plasma and blood USL311 concentrations concentration-dependent manner. Based on these observations, a longer duration of USL311 infusion (i.e., slower infusion rate) was expected to decrease the magnitude of QT-prolongation and therefore as of Amendment #3 the the infusion duration was increased from 120 minutes (2 hours) to 240 minutes (4 hours).

Dosing with the 4 hour infusion was initiated at the highest safe dose of USL311 as determined from subjects who received USL311 via a 2 hour infusion (viz., prior to Amendment #3 Subjects who were previously enrolled and are actively participating at the time Protocol Amendment #3 was activated continued study participation according to Protocol Amendment #2.

Dose-escalation was to occur as described in criteria outlined in Section 3.2.1 and Section 3.2.3. All dose-escalation decisions will be determined by the DEC (see Section 3.2.5).

Part 1b – Single Agent Oral USL311 Dose Escalation:

After 13 subjects were evaluable (i.e., had completed Cycle 1 dosing, at minimum) in Part 1a IV Dose Escalation, the DEC halted dose escalation, although no DLTs had occurred, due to continuing dose-related increases in QTcF prolongation that was not ameliorated by increasing the infusion duration. Part 1b of the study will resume under this amendment with dosing initiation of an oral formulation of USL311, at a starting dose of 40 mg once daily in 3-week (21-day) cycles.

Dose escalation will begin in the single agent oral formulation with enrollment of subjects to the 40 mg dose. Treatment will be administered once daily in 3-week (21-day) cycles. USL311 will be administered as oral tablets once daily in the morning under fasted conditions for 2 hours before and 2 hours after the dose on Cycle 1-Days 1, 2, 8, and 15 and on Cycle 2-Day 1. For all other outpatient dosing days, the subject should be instructed to make an effort to take the oral tablets at least 1 hour before or at least 1 hour after their meal, and they will be instructed to document in their diary the time of any food eaten prior to dosing and for 1 hour after dosing. It is recommended that subjects take their dose at the same time each day, except for visit days, when the dose will be held and taken during the clinic visit.

The selected 40 mg daily oral starting dose is comparable to the 30 mg daily starting dose based on 1/6 HNSTD in dogs (5 mg/kg/day oral daily dose) and is lower than the ~60 mg daily dose calculated based on the maximum IV dose tested in humans (250 mg/m^2 weekly, divided into daily doses). USL311 plasma pharmacokinetics are dose-proportional and minimal to no plasma accumulation is expected for USL311 and its main metabolites after daily oral dosing. In addition, a fixed-dosing approach is considered to be appropriate for future studies given that USL311 pharmacokinetics are not influenced by body size. Daily oral dosing is anticipated to further mitigate the concentration-related QT effect, compared to IV bolus weekly dosing.

Part 2 – USL311 in Combination with Lomustine Dose Escalation**:**

The starting dose of lomustine will be 90 mg/m^2. The starting dose for lomustine was selected based on the anticipated tolerability of the dose based on doses typically used in clinical practice. The maximum lomustine dose allowed during dose escalation is 130 mg/m^2 which is the maximum dose allowed per the package insert (see Appendix 4). The starting **dose** of oral USL311 used in combination with lomustine in Part 2 will be established following determination of the MTD of USL311 in Part 1b. The starting dose will be no higher than 2 evaluated dose levels below the MTD determined in Part 1. The dose levels will be determined by those doses of USL311 evaluated in Part 1b.

Dose-escalation will occur as described in criteria outlined in Section 3.2.2 and Section 3.2.3. All dose-escalation decisions will be determined by the DEC (see Section 3.2.4).

6.1.2.2. Phase 2

Part 3 – Single Agent USL311 Dose Expansion in Relapsed/Recurrent GBM:

The RP2D for single-agent USL311 that will be evaluated in Part 3 will be equal to, or lower than, the MTD determined in Study Part 1b.

Part 4 – USL311 in Combination with Lomustine Dose Expansion in Relapsed/Recurrent GBM:

The RP2D regimen for USL311 in combination with lomustine that will be evaluated in Part 4 will be equal to, or lower than, the MTD for the combination determined in Study Part 2.

6.1.3. Risks and Toxicities

6.1.3.1. USL311

As this study will be the first-in-human use of USL311, predictions of potential clinical toxicities are based on the animal toxicology studies. Potential toxicities identified from the animal toxicology studies, using much higher doses than the planned doses to be studied in the clinical human studies, include drug-induced phospholipidosis (DIPL), QTc interval prolongation, and tremors. Details from the animal toxicology studies are describe in Section 1.2.4 and IB.

DIPL is the excessive accumulation of phosopholipids and drug in lysosomes. It is unclear of the significance of toxicity associated with DIPL, and it is observed in multiple marketed agents (53). DIPL will be measured through an exploratory biomarker di-docosahexaenoyl (22:6)-bis(monoacylglycerol) phosphate (BMP) described in Section 8.7.3.

DIPL is often associated with QTc prolongation (54) as was observed in the canine nonclinical toxicology studies. QTc prolongation may increase the risk of arrhythmias, most significantly Torsades de Pointes (TdP); however, no a rrhythmias were observed in the nonclinical toxicology studies. QTc prolongation has been observed in Part 1a of the clinical study. Cardiac monitoring will be used during the dosing of study drug(s) as described in Section 8.4.7.

In the nonclinical studies, 4-AP was identified as a metabolite of USL311 (see Section 1.2.3.2). 4-AP (46) is a marketed drug approved as a treatment in Multiple Sclerosis. 4-AP is associated with increased risk of seizure activity above a likely threshold plasma concentration of about 100 ng/ml (47). It is not expected that the plasma concentrations of 4-AP will approach the threshold concentration at the starting dose of USL311 and subsequent doses during dose escalation and the RP2D. The 4-AP plasma concentration will be measured as part of the PK assessment (see Section 8.6.1).

Similar in class agents include plerixafor (55) which is a CXCR4 partial agonist. Common toxicities occurring ≥ 10% of patients include diarrhea, nausea, fatigue, injections site reactions, headache, arthralgia, dizziness, and vomiting.

It is not known whether USL311 causes phototoxicity. However, subjects receiving USL311 should be cautioned to avoid unprotected sun exposure.

6.1.3.2. Lomustine

Bone marrow suppression, notably thrombocytopenia and leukopenia, is the most common and severe of the toxic effects of lomustine. Blood counts will be monitored at each treatment visit to monitor for bone marrow suppression. At the recommended dosage, courses of lomustine should not be given more frequently than every 6 weeks. The bone marrow toxicity of

lomustine is cumulative and therefore dosage adjustment must be considered on the basis of nadir blood counts from prior dose. Dose modifications will occur due to toxicity as described in Section 6.1.5.2.

Lomustine is listed a moderate-to-high emetogenic drug, thus nausea and vomiting may occur with lomustine administration. Subjects will be able to take prophylactic antiemetic agents, per ASCO guidelines (51) and the discretion of the Investigator. For a list of permitted antiemetics see Section 5.3.1.

For more information about toxicities see Appendix 4 for the lomustine (Gleostine®) package insert.

6.1.4. Monitoring and Toxicity Management

Each subject receiving USL311 alone, or in combination with lomustine, will be evaluable for safety at each study visit. Toxicity will be assessed according to the NCI CTCAE version 4.03. Dose adjustments will be made according to the system showing the greatest degree of toxicity or the toxicity of greatest concern.

6.1.5. Dose Modifications

6.1.5.1. USL311 Dose Modifications

Dosing of USL311 may be interrupted to allow for recovery from toxicity, with IV doses delayed for up to 14 days beyond the scheduled dosing day or daily dosing held for up to 14 days. Thereafter, treatment at the same or a reduced USL311 dose can be considered, based upon discussions between Sponsor or designee and Investigator, if the subject has not developed progressive disease. Subjects with toxicities that require interruptions of greater than 14 days should be discontinued from the study. During Part 2 and Part 4, the combination of USL311 and lomustine will be considered, and the most likely agent(s) responsible for the observed toxicity will be modified. Dose modifications should be made based on observed toxicity as follows:

- Grade 1 or 2 toxicity: No requirement for dose interruption or dose reduction. If the toxicity persists at grade 2, a dose reduction to the next lower dose level may be implemented at the discretion of the Investigator.
- Grade 3 toxicity: Dosing should be stopped. USL311 dosing may resume at the next lower dose level when toxicity resolves to grade 1 or returns to baseline.
- Grade 4 toxicity: Dosing should be stopped. USL311 may resume at a lower dose level (1-2 dose level decrease) with the approval of the medical monitor when toxicity resolves to grade 1 or returns to baseline.

USL311 will be permanently discontinued for individual subjects as a result of any unresolved Grade 3 or Grade 4 toxicity or based on a decision by the subject or Investigator that continued USL311 treatment is not in the subject's best interest.

USL311 dose modification criteria based on ECG assessments:

- To determine eligibility to initiate treatment, the last safety ECG performed prior to the first administration of study drug(s) will be used as baseline. The baseline QTcF interval must be ≤ 450 msec before the first dose of study drug(s). If the baseline QTcF interval is > 450 msec, the subject will not be eligible for the study.
- On subsequent doses, the predose safety ECG on the day of dosing will be used to determine eligibility for dosing. Subjects will be eligible to receive the dose of USL311 if the predose QTcF interval is < 501 msec, per Investigator discretion.
- If any post-dose QTcF interval is ≥ 501 msec, repeat ECG assessments will occur at least every 30 minutes until the QTcF interval is < 501 msec.
- USL311 dosing may be interrupted, per Investigator discretion, based on findings from real-time bedside ECG or scheduled and unscheduled ECG assessments.

6.1.5.2. Lomustine Dose Modifications

Lomustine dose modifications will be based on lomustine prescribing information (see Appendix 4). Doses subsequent to the initial dose should be adjusted according to the hematologic response of the subject to the preceding dose. The following schedule (Table 18) is suggested as a guide to dosage adjustments:

Table 18: Lomustine Dosage Adjustment Guide

Nadir After Prior Dose		Percentage of Prior Dose to be Given
Leukocytes (/mm^3)	Platelets (/mm^3)	
≥4000	≥100,000	100%
3000 – 3999	75,000 – 99,999	100%
2000 – 2999	25,000 – 74,999	70%
<2000	<25,000	50%

A repeat course of lomustine should not be given until circulating blood elements have returned to acceptable levels (platelets above 100,000/mm^3; leukocytes above 4000/mm^3), and this is usually in 6 weeks. Blood counts should be monitored weekly and repeat courses should not be given before 6 weeks because the hematologic toxicity is delayed and cumulative.

The use of granulocyte growth factor support will be consistent with the ASCO guidelines (52).

Lomustine will be permanently discontinued for individual subjects as a result of any unresolved Grade 3 or Grade 4 toxicity or based on a decision by the subject or Investigator that continued lomustine treatment is not in the subject's best interest.

6.1.6. Duration of Treatment

Subjects who tolerate Cycle 1 treatment and do not have progressive disease, treatment failure or relapse are eligible to continue to receive treatment until they experience unacceptable toxicity, disease progression, meet any of the withdrawal criteria, or the study is terminated by the Sponsor.

6.1.7. Treatment Compliance

During study visits for all parts of the study, f USL311 (IV or oral) will be administered under the supervision of qualified study center personnel. As IV study drug is administered by qualified study center personnel in Part 1a, treatment compliance is expected to be 100%.

For Parts 1b, 2, 3 and 4, at each weekly visit (i.e., Day 1, Day 8, Day 15, etc.), USL311 tablets will be dispensed from a bulk supply, returned drug will be counted and compliance calculated. USL311 will be administered in the clinic on each visit day; on all other days it will be self-administered as an outpatient by the subject and documented in a patient diary. The diary will be brought to each weekly visit for review and reconciliation against the pill counts to confirm that the diary is being completed accurately. If compliance drops below 70%, patients will be re-educated on t he need for remaining compliant with daily dosing. Intentional non-compliance could be cause for discontinuation from the study.

Lomustine will be administered in the clinic for Part 2 on Cycle 1-Day 1 and Cycle 2-Day 2 and for Part 4 on C ycle 1-Day 2. Lomustine will be administered under the supervision of qualified study center personnel and treatment compliance is expected to be 100% for these doses. For all other cycles, lomustine will be administered on Day 2 of the cycle which may occur on an outpatient basis if allowed per site standard of practice. If lomustine is administered as an outpatient dose, clinic staff will follow-up by phone with the subject within 48 hours after Day 2 to collect the following: time and date of lomustine administration, concomitant medications, and AEs. If lomustine is administered as an outpatient dose, compliance will be captured through these follow-up phone calls.

The allowable dosing windows are defined in the footnotes of Table 1 – Table 7. Dosing outside of the windows requires approval based upon discussion between Sponsor or designee and Investigator.

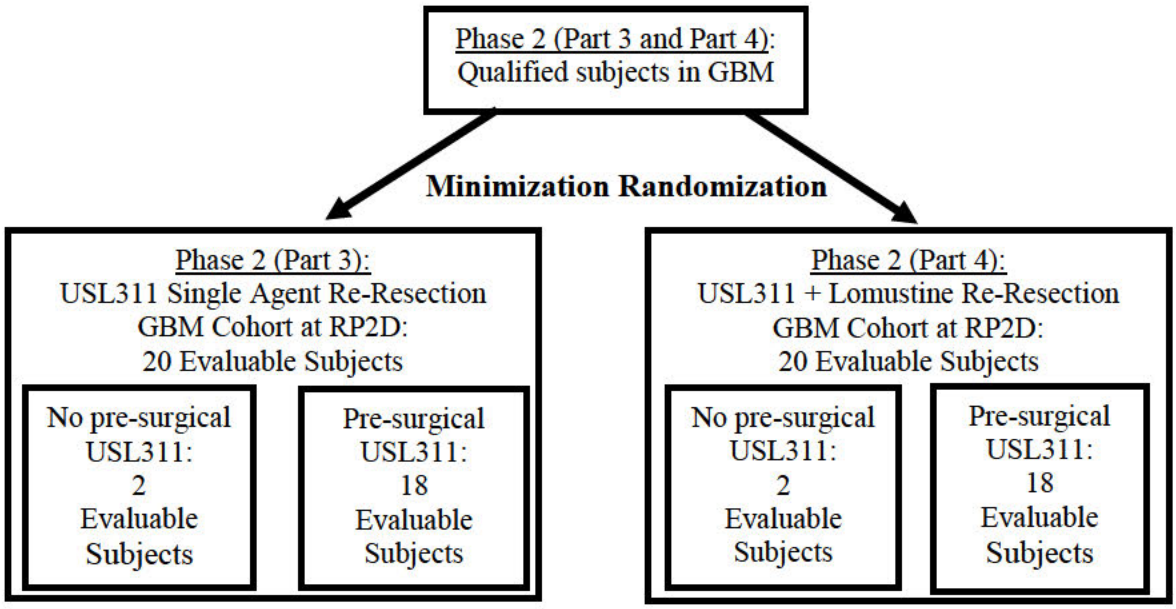
6.2. Treatment Randomization

Randomization will not occur in the Phase 1 dose-escalation portion (Parts 1 and 2). Subjects in the Phase 2 dose-expansion (Parts 3 and 4) in relapsed/recurrent GBM will be randomized after the Screening visit to one of the four possible treatments shown in Figure 3.

Subjects may begin enrolling into Part 3 (N = 20) after the completion of enrollment in Part 1 and determination of the RP2D for single agent USL311 and subjects may begin enrolling into Part 4 (N = 20) after the completion of enrollment in Part 2 and the determination of the RP2D for USL311 in combination with lomustine. In Parts 3 and 4, 18 subjects in each part will be randomized to receive pre-surgical USL311 treatment and 2 subjects in each part to receive no pre-surgical USL311 treatment. All subjects in Part 3 will receive post-surgical USL311 treatment, whereas all subjects in Part 4 will receive post-surgery USL311 treatment in combination with lomustine.

Due to the potentially staggered start of Parts 3 a nd 4, a minimization randomization mechanism will be used to assign subjects to Phase 2 treatment in order to obtain a target of approximately 40 response-evaluable subjects at the end of the study. Minimization is considered to be an appropriate randomization method for this type of scenario to ensure well-balanced randomization between treatments.

Figure 3: Phase 2 Dose-Expansion in Relapsed/Recurrent GBM: Treatment Allocation



6.3. Treatment Blinding

This is an open-label study. No blinding will be performed.

6.4. Study Drug Identification and Supply

Sponsor and/or its designee will provide the study site with an initial supply and replenishments of USL311 and lomustine sufficient for the completion of the study.

USL311 Injection Solution is supplied as a 50 mg/mL sterile intravenous (IV) solution of USL311. The formulation contains 50 m M citrate buffer at pH 3.5. A single packaging configuration is supplied containing a 12-mL fill in a 20-mL crimp sealed vial.

Oral USL311 is supplied as 20 mg or 100 mg tablets for oral administration. The inactive ingredients are microcrystalline cellulose, lactose, crospovidone, and magnesium stearate. Both tablet dose strengths are smooth, round, concave tablets containing 25% USL311 by weight and film coated with 3% Opadry white without any visible markings (embossing or debossing). Thirty (30) tablets are packaged in each 75 cc high density polyethylene bottle with a child resistant screw cap.

Lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea, CCNU) is available in 10 m g, 40 mg, and 100 mg capsules for oral administration. Inactive ingredients in lomustine capsules are magnesium stearate and mannitol. Refer to Appendix 4 for the lomustine (Gleostine®) package insert.

6.5. Packaging and Labeling

USL311 Injection Solution and tablets, and lomustine will be shipped to an authorized drug distribution company for labeling and packaging.

The study drug supplies will be labeled appropriate to their use. USL311 and lomustine will be identified as unblinded treatments. The USL311 label will contain at a minimum the following information:

- Legal requirements as specified by country-specific regulations
  - Protocol number
  - Instructions for use (if required)
  - Name and address of sponsor
  - Quantity/contents
  - Batch/Lot number
  - Storage conditions
  - Expiration date, as applicable
  - "Caution: New Drug – Limited by Federal law to investigational use" will also appear on the immediate package of vial used during the study as required by 21 CFR §312.6, as applicable
  - Each container of study drug will be clearly labeled with study-specific information meeting all the applicable regulatory requirements.

Clinical study drug supplies will be labeled in the official language of the country of the study site, if applicable.

6.6. Handling, Storage and Accountability

USL311 Injection Solution is stable for the lot life indicated on the package labeling when stored refrigerated at 2-8°C (36-46°F). After admixing, the admixed product may be stored for no more than **6** hours at room temperature (15-30°C or 59-86°F) or no more than 24 hours under refrigeration (2-8°C or 36-46°F). Administration of the admixed product must be complete within 24 hours.

USL311 tablets will be stored between 20-25°C. Documentation of shipment inventory including expiration date will be provided to each site on the shipping inventory form. Tablets will be dispensed in a child resistant container and will include dosing instructions. Lomustine capsules are stable for the lot life indicated on package labeling when stored in well-closed containers at room temperature (25°C [77°F]); excursions permitted to 15–30°C (59–86°F). Avoid excessive heat (over 40°C, 104°F). Caution should be exercised when handling lomustine capsules. It is recommended that individuals handling lomustine wear gloves for protection. Refer to Appendix 4 for the lomustine package insert and special handling procedures.

The Sponsor requires Investigators or designee to maintain adequate drug inventory and security at all times. Upon receipt of the study drug, the Investigator (or his/her designee) will perform an inventory of the shipment, comparing the shipment inventory to actual study drug received, and complete and sign an inventory log. The Investigator (or his/her designee) must count and verify that the shipment contains all the items that appear on the shipment inventory.

The Investigator must immediately notify the Sponsor (or its designee) or the drug-distribution contractor of any damaged or unusable study drug that the study center receives and document any damaged or unusable study drug in the inventory log.

Only after receipt of all required documentation from a study center will the Sponsor (or its designee) notify the drug distribution contractor to distribute the initial study drug to that study center. Additional study drug will be shipped as needed to the study center. The Investigator (or his/her designee) will retain in the study file a copy of the shipment inventory received with the study drug supply. Each time study drug is dispensed to a subject, the Investigator (or his/her designee) will record the quantity and a description (e.g., subject number) on the drug accountability log. The Investigator (or his/her designee) will also document any subsequent returns or losses of study drug on the drug accountability log.

Drug-accountability records will be available to the study monitor for review at each study-center visit. The study monitor will inspect drug supplies and accountability records throughout conduct of the study at the study center to confirm inventory control and proper study drug storage. The study monitor will record any discrepancies and/or deficiencies and report them to the Investigator and to the Sponsor (or its designee) and will document the Investigator's plan for resolution of any drug inventory or storage issues.

6.6.1. Dispensing of Study Drug

All study drugs will be dispensed by qualified study-center personnel and will be documented in the drug accountability log.

USL311 20 mg and 100 mg tablets will be dispensed in adequate quantities based on the subject's assigned daily dose. Separate child-resistant containers should be used for 20 mg tablets and 100 mg tablets.

At Cycle 1-Day 1, a 10-day supply of USL311 tablets (to cover the potential visit window +3 days) will be dispensed to the subject, along with a patient diary, which will be maintained by each subject to track their administration of study drug; the patient will be trained on the proper use of the diary and will be instructed to make an entry each day after taking their dose of USL311. Subjects will also be instructed to bring their remaining medication and their diaries to each study visit, and to refrain from taking their USL311 dose on the days of clinic visits, in particular for return visits on Days 2, 3 and 5 of Cycle 1. Subjects will be dosed during clinic visits from their own supply of USL311. After reviewing the diary for accountability, a new supply of study drug should be dispensed to replace administered drug such that the subject again has a 10-day supply of USL311 tablets. In the event that the subject fails to bring their medication with them to the clinic, a 10-day supply should be dispensed from the pharmacy, and the subject's in-clinic dose should be administered from this supply.

For lomustine specifically, doses will be administered in the clinic for Part 2 on Cycle 1-Day 1 and Cycle 2-Day 2 and for Part 4 on Cycle 1-Day 2. For all other cycles, lomustine will be administered on Day 2 of the cycle which may occur on an outpatient basis if allowed per site standard of practice. The outpatient doses will be dispensed during the Day 1 Visit of the cycle. If lomustine will be administered in the clinic, the dose will be dispensed on the dosing day.

6.6.2. Return and Disposition of Clinical Supplies

At the conclusion of the study, a final inventory of study drug shipped to, dispensed by, and remaining at the study center will be performed by the clinical research associate (CRA). This reconciliation will be logged on the drug accountability form, and the form will be signed and dated. If any supplies are missing, this discrepancy must be indicated on the drug accountability/ return forms along with an explanation of the discrepancy. Any discrepancies noted will be investigated, resolved, and documented before return or destruction of unopened study drug. The Investigator (or his/her designee) must return all unopened study drug to the Sponsor (or its designee) unless alternative arrangements for drug disposal are authorized by the Sponsor. A copy of the drug-accountability records should be sent to the Sponsor (or its designee), and the Investigator (or his/her designee) must retain the originals of these drug-accountability records for his/her files in accordance with 21 CFR § 312.59.

No study drug will be retained at any study center after the study is completed; all study drugs will be returned to the Sponsor (or its designee) for destruction or destroyed on site according to regulations, as applicable.

7. STUDY VISIT SCHEDULE AND ASSESSMENTS

Tables for the schedule of visits and timing of assessments are detailed in Study Schedule of Visits and Assessments, Table 1 – Table 7. Only assessments required for the study are listed in the tables, any additional assessments or repeat assessments should be performed as indicated and according to clinical practice.

All study assessments should be performed at the visits and nominal time points outlined in the tables found in the Study Schedule of Visits and Assessments, Table 1 – Table 7. Actual completion date and times will be recorded in the source document and eCRF. Other logistical considerations (e.g., order of events and assessment windows) will be outlined in study-specific procedures found in the footnotes of the Study Schedules of Visits and Assessments.

7.1. Screening Phase

All subjects will provide written informed consent before any study-specific procedure is performed.

During screening, subjects will undergo assessments to determine if they are eligible to participate in the study according to the inclusion/exclusion criteria. In Parts 1 and 2, Screening Visit assessments must be performed within 28 days of Cycle 1-Day 1, and in Parts 3 and 4, within 28 days of Pre-surgical Dosing, Day 1 for those randomized to pre-surgical USL311 treatment and within 28 days of Surgery for those randomized to no pre-surgical treatment. If the Screening Visit falls outside of the 28-day window prior to the first Treatment Phase day, the subject will be allowed to complete the Screening Visit assessments again. The repeated Screening Visit will be used to determine eligibility.

All Screening Visit assessments must be performed, and subjects must meet eligibility requirements prior to the subject entering the Treatment Phase. Any screening results falling outside of the reference ranges may be repeated at the discretion of the Investigator.

The screening assessments will be performed in each study part unless specified below.

Screening Visit:

The following assessments will be performed during the Screening Visit:

- Obtain informed consent of subject or assent of subject, if applicable (see Section 5.8)
- Assessment of inclusion/exclusion criteria (see Section 5.2)
- Collect medical history (see Section 8.4.2)
- Collect oncologic history (see Section 8.4.3)
- Physical examination (see Section 8.4.5.1)
- Neurological examination (see Section 8.4.5.2)
- Vital signs (see Section 8.4.6)
- Collect KPS (see Section 8.4.1)

- Safety ECG (Parts 1 and 2 only) or Extracted ECG (Part 3 and 4 only) (see Section 8.4.7)
- Blood sample collection for serum pregnancy test for all females of child bearing potential (see Section 8.4.9)
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
- Urine sample collection for UA (see Section 8.4.8)
- Tumor assessment by RECIST v.1.1 criteria (Parts 1 and 2 only; see Section 8.2.2) or by RANO criteria (see Section 8.2.3) as appropriate
- Collect adverse events occurring after written informed consent is obtained (see Section 9.1.1)
- Collect prior and concomitant medication information (see Section 8.4.4)
- Confirm availability of archival tumor tissue (see Section 8.4.10)
- Perform EORTC QLQ-C30/BN20 (Parts 3 and 4 only; see Section 8.7.1)

Baseline Visit (Day -1, Part 1 Only):

The following assessments will be performed during the Baseline Visit (Day -1) in Part 1 only:

- Assessment of inclusion/exclusion criteria (see Section 5.2)
- Physical examination (see Section 8.4.5.1)
- Vital signs (see Section 8.4.6)
- Collect KPS (see Section 8.4.1)
- Holter-extracted ECG (see Section 8.4.7)
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
- Blood for clinical laboratory tests (see Section 8.4.8)
- Blood sample for cytochrome P450 genotyping (see Section 8.4.8.2)
- Blood sample for optional pharmacogenomics analyses (see Section 8.4.8.3)
  - **Note**: May be collected at a later visit if determined appropriate per the clinical site
- Urine sample collection for UA (see Section 8.4.8)
- Adverse event monitoring (see Section 9.1.1)
- Concomitant medication monitoring (see Section 8.4.4)
- Urine sample collection for BMP analysis (see Section 8.7.3)

The following screening safety assessments are required to be repeated at Baseline (Day -1) only for subjects for whom screening assessments were performed > 3 days before

Cycle 1-Day 1 (1st dose of USL311): inclusion/exclusion criteria, physical examinations, UA, and clinical laboratory tests. If collected ≤ 3 days prior to Cycle 1-Day 1, these assessments do not need to be repeated.

7.2. Treatment Phase

Following the completion of the Screening Phase, subject will be eligible to enter into the Treatment Phase of the study.

7.2.1. Part 1a Treatment

Subjects enter the Treatment Phase in Part 1 on Cycle 1-Day 1. Treatment with single agent USL311 will be administered in 21-day cycles.

Cycle 1 Visits:

The following assessments will be performed during Cycle 1:

- USL311 IV administration in the clinic (see Section 6.1.1.3)
  - Day 1, Day 8, and Day 15
- Physical examination (see Section 8.4.5.1)
  - Day 8 and Day 15
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, and Day 15
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, and Day 15
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 8, and Day 15
- Safety ECG (see Section 8.4.7)
  - Day 1, Day 8 and Day 15
- Real-time bedside ECG (see Section 8.4.7)
  - Day 1, Day 8, and Day 15
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 8 and Day 15
- Adverse events monitoring (see Section 9.1.1)
  - Continuous through Cycle 1
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 1

- Blood sample collection for USL311 PK (see Section 8.6.1; Table 8)
  - Day 1, Day 2, Day 3, Day 5, Day 8, and Day 15
- Blood sample collection for CD34+ cell count (see Section 8.7.3; Table 8)
  - Day 1 and Day 2
- Blood sample collection for WBC count (see Section 8.7.3; Table 8)
  - Day 1, Day 2, Day 3, Day 5, Day 8, and Day 15
- Blood samples for SDF-1 analysis (see Section 8.7.3; Table 8)

Day 1 and Day 2Visits for Part 1a, Cycle 1 are on Day 1, Day 2, Day 3, Day 5, Day 8 and Day 15. See Table 1 for the schedule of events on each day of the cycle and timing of assessments. During Cycle 1, the Baseline (Day -1), Day 1 and Day 2 visits will occur on consecutive days, whereas Day 3 and Day 5 assessments (PK and PD samples) will be collected according to the acceptable time windows specified in Table 8. All remaining Cycle 1 visits will occur on the scheduled study day or ≤ 3 days post the scheduled study day.

Cycle 2+ Visits:

The following assessments will be performed during Cycle 2 and all subsequent cycles:

- USL311 IV administration (see Section 6.1.1.3)
  - Day 1, Day 8, and Day 15
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, and Day 15
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, and Day 15
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, and Day 15
- Collect KPS (see Section 8.4.1)
  - Day 1 of even numbered cycles only
- Holter-extracted ECG (see Section 8.4.7)
  - If clinically indicated from experience in Cycle 1
- Safety ECG (see Section 8.4.7)
  - If clinically indicated from experience in Cycle 1
- Real-time bedside ECG (see Section 8.4.7)
  - If clinically indicated from experience in Cycle 1
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)

  - Day 1 of even numbered cycles only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, and Day 15
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Tumor assessment by RECIST v.1.1 criteria (see Section 8.2.2) or by RANO criteria (see Section 8.2.3) as appropriate
  - Cycle 3-Day 1 then every 6 weeks (± 7 days) thereafter
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 2+
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 2+
- Blood sample collection for USL311 PK (see Section 8.6.1; Table 8)
  - Day 1, Cycle 2 only
- Blood sample collection for WBC count (see Section 8.7.3; Table 8)
  - Day 1, Cycle 2 only
- Urine sample collection for BMP analysis (see Section 8.7.3)
  - Day 1 of even-numbered cycles only

The study visits for Part 1a, Cycle 2+ are on Day 1, Day 8 and Day 15. See Table 1 for the schedule of events on each day of the cycle and timing of assessments. For all visits in Cycle 2+ (up to but not including the End of Treatment Visit), visits will occur a minimum of 7 days and a maximum of 14 days (unless treatment is on hold for safety reasons) from the previous dose of USL311.

7.2.2. Part 1b Treatment

Subjects enter the Treatment Phase in Part 1b on Cycle 1-Day 1. Treatment with single agent USL311 will be administered in 21-day cycles.

Cycle 1 Visits:

The following assessments will be performed during Cycle 1:

- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8, and Day 15
- USL311 administration in the clinic (see Section 6.1.1.4)
  - Day 1, Day 2, Day 8, and Day 15

- Physical examination (see Section 8.4.5.1)
  - Day 8 and Day 15
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, and Day 15
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, and Day 15
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 8, and Day 15
- Safety ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 8 and Day 15
- Real-time bedside ECG (see Section 8.4.7)
  - Day 1, Day 8, and Day 15
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 8 and Day 15
- Adverse events monitoring (see Section 9.1.1)
  - Continuous through Cycle 1
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 1
- Blood sample collection for USL311 PK (see Section 8.6.1; Table 9)
  - Day 1, Day 2, Day 8, and Day 15
- Blood sample collection for CD34+ cell count (see Section 8.7.3; Table 9)
  - Day 1 and Day 2
- Blood sample collection for WBC count (see Section 8.7.3; Table 9)
  - Day 1, Day 2, Day 8, and Day 15

Visits for Part 1b, Cycle 1 are on Day 1, Day 2, Day 8 and Day 15. See Table 2 for the schedule of events on each day of the cycle and timing of assessments. During Cycle 1, the Baseline (Day -1), Day 1 and Day 2 visits will occur on consecutive days. All remaining Cycle 1 visits will occur on the scheduled study day or ≤ 3 days post the scheduled study day.

Cycle 2+ Visits:

The following assessments will be performed during Cycle 2 and all subsequent cycles:

- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8, and Day 15

- USL311 administration in the clinic (see Section 6.1.1.4)
  - Day 1, Day 8 and Day 15
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, and Day 15
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, and Day 15
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, and Day 15
- Collect KPS (see Section 8.4.1)
  - Day 1 of even numbered cycles only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, then only if clinically indicated
- Safety ECG (see Section 8.4.7)
  - Day 1, then only if clinically indicated
- Real-time bedside ECG (see Section 8.4.7)
  - If clinically indicated from experience in Cycle 1
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 of even numbered cycles only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, and Day 15
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Tumor assessment by RECIST v.1.1 criteria (see Section 8.2.2) or by RANO criteria (see Section 8.2.3) as appropriate
  - Cycle 3-Day 1 then every 6 weeks (± 7 days) thereafter
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 2+
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 2+

- Blood sample collection for USL311 PK (see Section 8.6.1; Table 9)
  - Day 1, Cycle 2 only
- Blood sample collection for WBC count (see Section 8.7.3; Table 9)
  - Day 1, Cycle 2 only
- Urine sample collection for BMP analysis (see Section 8.7.3)
  - Day 1 of even numbered cycles only

The study visits for Part 1b, Cycle 2+ are on Day 1, Day 8 and Day 15. See Table 2 for the schedule of events on each day of the cycle and timing of assessments. For all visits in Cycle 2+ (up to but not including the End of Treatment visit), visits will occur within ±3 days of the scheduled visit.

7.2.3. Part 2 Treatment

Subjects enter the Treatment Phase in Part 2 on the first day of study drug(s) administration. Treatment with USL311 in combination with lomustine will be administered in 42-day cycles, except in Cycle 1 which will be a 49-day cycle. Screening safety assessments are only required to be repeated at Cycle 1-Day 1 (baseline) for subjects for whom screening assessments were performed > 3 days before Cycle 1-Day 1 (1st dose of lomustine): inclusion/exclusion criteria, physical examinations, UA, and clinical laboratory tests. If collected ≤ 3 days prior to Cycle 1-Day 1, these assessments do not need to be repeated and the screening results will be considered baseline.

Cycle 1 Visits:

The following assessments will be performed during Cycle 1:

- Assessment of inclusion/exclusion criteria (see Section 5.2)
  - Day 1 only
- Lomustine administration (see Section 6.1.1.5)
  - Day 1 only
- USL311 dispensing (see Section 6.6.1)
  - Day 8, Day 15, Day 22, Day 29, Day 36, and Day 43
- USL311 administration in-clinic (see Section 6.1.1.4)
  - Day 8, Day 9, Day 15, Day 22, Day 29, Day 36, and Day 43
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, Day 36, and Day 43
- Neurological examination (see Section 8.4.5.2)
  - Day 8, Day 15, Day 22, Day 29, Day 36, and Day 43

- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, Day 15, Day 22, Day 29, Day 36, and Day 43
- Collect KPS (see Section 8.4.1)
  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, Day 8, Day 9, Day 15, Day 22, Day 29, Day 36, and Day 43
- Safety ECG (see Section 8.4.7)
  - Day 1, Day 8, Day 9, Day 15, Day 22, Day 29, Day 36, and Day 43
- Real-time bedside ECG (see Section 8.4.7)
  - Day 8, Day 15, Day 22, Day 29, Day 36, and Day 43
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, Day 15, Day 22, Day 29, Day 36, and Day 43
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Blood sample for cytochrome P450 genotyping (see Section 8.4.8.2)
  - Day 1 only
- Blood sample for optional pharmacogenomics analyses (see Section 8.4.8.3)
  - Day 1 or may be collected at a later visit if determined appropriate per the clinical site
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 1
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 1
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 10)
  - Day 8, Day 9, Day 15 and Day 22
- Blood sample collection for lomustine PK analysis (see Section 8.6.1; Table 10)
  - Day 1 only

- Blood sample collection for CD34+ cell count (see Section 8.7.3; Table 10)
  - Day 8, Day 9, Day 15 and Day 22
- Blood sample collection for WBC count (see Section 8.7.3; Table 10)
  - Day 8, Day 9, Day 15 and Day 22
- Urine sample collection for BMP analysis (see Section 8.7.3)
  - Day 1 only

The study visits for Part 2 Cycle 1 are on Day 1, Day 8, Day 9, Day 15, Day 22, Day 29, Day 36, and Day 43. See Table 3 for the schedule of events on each day of the cycle and timing of assessments. During Cycle 1-Day 8 and Day 9 visits will occur on consecutive days. All remaining Cycle 1 visits will occur on the scheduled study day or ≤ 3 days post the scheduled study day.

Cycle 2+ Visits:

The following assessments will be performed during Cycle 2 and all subsequent cycles:

- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- USL311 administration in the clinic (see Section 6.1.1.4)
  - Day 1, Day 2, Day 8, Day 15, Day 22, Day 29, and Day 36
- Lomustine administration (see Section 6.1.1.5)
  - Day 2 only
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Collect KPS (see Section 8.4.1)
  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, then if clinically indicated
- Safety ECG (see Section 8.4.7)
  - Day 1, then if clinically indicated

- Real-time bedside (see Section 8.4.7)
  - If clinically indicated from experience in Cycle 1
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Tumor assessment by RECIST v.1.1 criteria (see Section 8.2.2) or by RANO criteria as appropriate (see Section 8.2.3)
  - Cycle 2-Day 1 then every 6 weeks (± 7 days) thereafter
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 2+
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 2+
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 10)
  - Day 1 and Day 2, Cycle 2 only
- Blood sample collection for lomustine PK analysis (see Section 8.6.1; Table 10)
  - Day 2, Cycle 2 only
- Blood sample collection for WBC count (see Section 8.7.3; Table 10)
  - Day 1, Cycle 2 only
- Urine sample collection for BMP analysis (see Section 8.7.3)
  - Day 1 only

The study visits for Part 2 Cycle 2+ are on Day 1, Day 2 (Cycle 2 only, if lomustine is administered as an outpatient dose for future cycles), Day 8, Day 15, Day 22, Day 29, and Day 36. See Table 3 for the schedule of events on each day of the cycle and timing of assessments. For all visits in Cycle 2+ (up to but not including the End of Treatment Visit), visits will occur within ± 3 days of the scheduled visit.

7.2.4. Part 3 Treatment

Subjects enter the Treatment Phase in Part 3 on the first day of pre-surgical USL311 administration for those randomized to pre-surgical treatment or on the day of surgery for those randomized to no pre-surgical treatment. Post-surgical treatment with USL311 will be administered in 21-day cycles.

Screening safety assessments are required to be repeated at Pre-surgical Dosing, Day 1 (for those randomized to pre-surgical treatment) or Surgery (for those randomized to no pre-surgical treatment) only for subjects for whom screening assessments were performed > 3 days before Pre-surgical Dosing, Day 1 (for those randomized to pre-surgical treatment) or Surgery (for those randomized to no pre-surgical treatment): inclusion/exclusion criteria, physical examination, neurological examination, UA, and clinical laboratory tests. For those randomized to no pre-surgical treatment, ECG and vital signs will also be repeated. If collected ≤ 3 days prior to Pre-surgical Dosing, Day 1 (for those randomized to pre-surgical treatment) or Surgery (for those randomized to no pre-surgical treatment), these assessments do not need to be collected and screening results will be considered baseline

For those randomized to no pr e-surgical treatment, if the safety assessments need to be repeated, the pre-surgical physical examination and neurological examination performed as part of standard-of-care and performed closest to the day of surgery may be used as baseline only if it is not feasible to perform the physical and neurological examinations on the day of surgery.

Pre-surgical Dosing Visits:

The assessments within the Pre-surgical Dosing Visits only apply to subjects randomized to receive pre-surgical treatment with USL311. Subjects randomized to receive no U SL311 treatment prior to surgery will not participate in any of the assessments within the Pre-surgical Dosing period.

The following assessments will be performed during the Pre-surgical Dosing Visits:

- Assessment of inclusions/exclusion criteria (see Section 5.2)
  - Day 1 only
- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8+
- USL311 administration in-clinic (see Section 6.1.1.3)
  - Day 1, Day 2, Day 3, Day 5 and Day 8+
- Physical examination (see Section 8.4.5.1)
  - Day 1 and Day 8+
- Neurological examination (see Section 8.4.5.2)
  - Day 1 and Day 8+
- Vital signs (see Section 8.4.6)
  - Day 1 and Day 8+
- Collect KPS (see Section 8.4.1)
  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)

  - Day 1, Day 2, Day 3, Day 5 and Day 8+
- Real-time beside ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 3, Day 5 and Day 8+
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1 and Day 8+
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Pre-surgical Dosing
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Pre-surgical Dosing
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 11)
  - Day 1, Day 2, Day 3, Day 5, and Day 8

Subjects randomized to receive pre-surgical treatment with USL311 are required to receive at least one week of treatment prior to surgery, and may receive up to a maximum of three weeks of treatment with USL311 (at the single agent RP2D defined in Part 1) prior to surgery. The length of treatment received will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Surgical re-resection may occur at any time from Day 2 onwards. USL311 will be administered on a daily schedule until Surgery. Assessments within the Pre-surgical Dosing Visits that are pending at the time of surgery will no longer be required and will not be performed post-surgery.

The study visits for Pre-surgical Dosing for Part 3 are on Day 1, Day 2, Day 3, Day 5, and Day 8+. See Table 4 for the schedule of events on each day of the cycle and timing of assessments. During the Pre-surgical Dosing, Day 1 and Day 2 visits will occur on consecutive days, whereas Day 3, Day 5, and Day 8 assessments (PK samples; dosing on Day 8 if possible) will be collected according to the acceptable time windows specified in Table 11. All remaining Pre-Surgical Dosing Visits are dependent on the length of pre-surgical USL311 treatment received and will occur within ± 3 days of scheduled visit.

Surgery (Re-resection) Visit:

For subjects randomized to pre-surgical treatment with USL311, Surgery (re-resection) may occur at any time from Day 2 onwards. For subjects randomized to receive no USL311

treatment prior to surgery, Surgery (re-resection) will occur following the Screening Visit with the Screening Day -28 to Day -1 window calculated relative to day of surgery.

The following assessments or procedures will be performed during the day of Surgery (re resection) for only those subjects randomized to no pre-surgical treatment:

- Assessment of inclusions/exclusion criteria (see Section 5.2)
- Physical examination (see Section 8.4.5.1)
- Neurological examination (see Section 8.4.5.2)
- Vital signs (see Section 8.4.6)
- Collect KPS (see Section 8.4.1)
- Holter-extracted ECG (see Section 8.4.7)
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
- Urine sample collection for UA (see Section 8.4.8)

The following assessments or procedures will be performed during the day of Surgery (re-resection) for all subjects in Part 3:

- Perform re-resection surgery (see Section 3.3.3)
- Collect any adverse events (see Section 9.1.1)
- Collect concomitant medication information (see Section 8.4.4)
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 11)
- CSF sample collection (see Section 8.6.2)
- Resected brain tissue sample collection (see Section 8.7.2)
- Tumor assessment by RANO criteria (see Section 8.2.3)
  - Performed ≤ 72 hours after surgery with EORTC QLQ-C30/BN20. If initiation of treatment following surgery is delayed for 3-4 weeks, a r epeat baseline response assessment using the RANO criteria along with EORTC QLQ-C30/BN20 will be performed ≤ 7 days of study drug dosing.
- Perform EORTC QLQ-C30/BN20 (see Section 8.7.1)
  - Performed ≤ 72 hours after surgery with response assessment using RANO criteria. If initiation of treatment following surgery is delayed for 3-4 weeks, a repeat baseline EORTC QLQ-C30/BN20 along with response assessment using RANO criteria will be performed ≤ 7 days of study drug dosing.

See Table 5 (for those randomized to no pre-surgical treatment) for the schedule of events on the day of the surgery and timing of assessments for Part 3.

Cycle 1 Visits:

Following surgery, all subjects (including those randomized to no pre-surgical treatment) will initiate treatment of USL311. Cycle 1-Day 1 (post-surgery) will be initiated ≤ 28 days of Surgery, although treatment delays may be considered based upon di scussions between Sponsor or designee and Investigator. Subjects must have fully recovered from surgery, including surgical wound healing, prior to initiating therapy. Post-surgical treatment with single agent USL311 will be administered in 21-day cycles.

The following assessments will be performed during Cycle 1:

- Assessment of inclusions/exclusion criteria (see Section 5.2)
  - Day 1 only
- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8 and Day 15
- USL311 administration in-clinic (see Section 6.1.1.3)
  - Day 1, Day 2, Day 8, and Day 15
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, and Day 15
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, and Day 15
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, and Day 15
- Collect KPS (see Section 8.4.1)
  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 8 and Day 15
- Real-time bedside ECG (see Section 8.4.7)
  - Day 1 only
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, and Day 15
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only

- Blood sample for cytochrome P450 genotyping (see Section 8.4.8.2)
  - Day 1 only
- Blood sample for optional pharmacogenomics analyses (see Section 8.4.8.3)
  - Day 1 or may be collected at a later visit if determined appropriate per the clinical site
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 1
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 1
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 12)
  - Day 1, Day 2, Day 8, and Day 15

The study visits for Part 3 Cycle 1 are on Day 1, Day 2, Day 8 and Day 15. See Table 4 (for those randomized to pre-surgical treatment) and Table 5 (for those randomized to no pre-surgical treatment) for the schedule of events on each day of the cycle and timing of assessments. For Cycle 1-Day 1 and Day 2 visits will occur on consecutive days, whereas all remaining Cycle 1 visits should occur on the scheduled study day or ≤ 3 days post the scheduled study day.

Cycle 2+ Visits:

The following assessments will be performed during Cycle 2 and all subsequent cycles:

- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8 and Day 15
- USL311 administration in-clinic (see Section 6.1.1.3)
  - Day 1, Day 8, Day 15
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, Day 15
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, Day 15
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, Day 15
- Collect KPS (see Section 8.4.1)
  - Day 1 of even numbered cycles only

- Holter-extracted ECG (see Section 8.4.7)
  - Cycle 2-Day 1 then if clinically indicated
- Real-time bedside ECG (see Section 8.4.7)
  - If clinically indicated
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 of even numbered cycles only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, Day 15
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Tumor assessment by RANO criteria (see Section 8.2.3)
  - Cycle 3-Day 1 then every 6 weeks (± 7 days) thereafter and at 6 months (± 7 days)
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 2+
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 2+
- Perform EORTC QLQ-C30/BN20 (see Section 8.7.1)
  - Every 6 weeks (± 7 days) and at 6 months (± 7 days)

The study visits for Part 3 Cycle 2+ are on Day 1, Day 8 and Day 15. See Table 4 (for those randomized to pre-surgical treatment) and Table 5 (for those randomized to no pre-surgical treatment) for the schedule of events on each day of the cycle and timing of assessments. For all visits in Cycle 2+ (up to but not including the End of Treatment Visit), visits will occur within ± 3 days of scheduled visit.

7.2.5. Part 4 Treatment

Subjects enter the Treatment Phase in Part 4 on t he first day of pre-surgical USL311 administration for those randomized to pre-surgical treatment or on the day of surgery for those randomized to no treatment. Post-surgical treatment with USL311 in combination with lomustine will be administered in 42-day cycles.

Screening safety assessments are required to be repeated at Pre-surgical Dosing, Day 1 (for those randomized to pre-surgical treatment) or Surgery (for those randomized to no pre-surgical treatment) only for subjects for whom screening assessments were performed > 3 days before Pre-surgical Dosing, Day 1 (for those randomized to pre-surgical treatment) or Surgery (for those randomized to no pre-surgical treatment): inclusion/exclusion criteria,

physical examination, neurological examination, UA, and clinical laboratory tests. For those randomized to no pre-surgical treatment, ECG and vital signs will also be repeated. If collected ≤ 3 days prior to Pre-surgical Dosing, Day 1 (for those randomized to pre-surgical treatment) or Surgery (for those randomized to no pre-surgical treatment), these assessments do not need to be collected and screening results will be considered baseline.

For those randomized to no pre-surgical treatment, if the safety assessments need to be repeated, the pre-surgical physical examination and neurological examination performed as part of standard-of-care and performed closest to the day of surgery may be used as baseline only if it is not feasible to perform the physical and neurological examinations on the day of surgery.

Pre-surgical Dosing Visits:

The assessments within the Pre-surgical Dosing only apply to subjects randomized to receive pre-surgical treatment with USL311. Subjects randomized to receive no USL311 treatment prior to surgery will not participate in any of the assessments within the Pre-surgical Dosing period.

The following assessments will be performed during the Pre-surgical Dosing Visits:

- Assessment of inclusions/exclusion criteria (see Section 5.2)
  - Day 1 only
- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8, then weekly thereafter
- USL311 administration in-clinic (see Section 6.1.1.3)
  - Day 1, Day 2, Day 3, Day 5 and Day 8, then weekly thereafter
- Physical examination (see Section 8.4.5.1)
  - Day 1 and Day 8, then weekly thereafter
- Neurological examination (see Section 8.4.5.2)
  - Day 1 and Day 8, then weekly thereafter
- Vital signs (see Section 8.4.6)
  - Day 1 and Day 8, then weekly thereafter
- Collect KPS (see Section 8.4.1)
  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 3, Day 5 and Day 8, then weekly thereafter
- Real-time bedside ECG (see Section 8.4.7)
  - Day 1 and Day 8, then weekly thereafter

- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1 and Day 8, then weekly thereafter
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Pre-surgical Dosing
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Pre-surgical Dosing
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 11)
  - Day 1, Day 2, Day 3, Day 5, and Day 8

Subjects randomized to receive pre-surgical treatment with USL311 are required to receive at least one week of treatment prior to surgery, and may receive up to a maximum of three weeks of treatment of USL311 (at the RP2D defined in Part 2) prior to surgery. The length of treatment received will depend on the surgery date. Scheduling of the surgery, as part of standard-of-care, should not be delayed due to the pre-surgical treatment. Surgical re-resection may occur at any time from Day 2 onwards. USL311 will be administered on a daily schedule until Surgery. Assessments within the Pre-surgical Dosing period that are pending at the time of surgery will no longer be required and will not be performed post-surgery.

The study visits for Pre-surgical Dosing for Part 4 are on Day 1, Day 2, Day 3, Day 5, and Day 8, then weekly thereafter. See Table 6 for the schedule of events on each day of the cycle and timing of assessments. During the Pre-surgical Dosing, Day 1 and Day 2 visits will occur on consecutive days, whereas Day 3, Day 5, and Day 8 assessments (PK samples) will be collected according to the acceptable time windows specified in Table 11. All remaining Pre-Surgical Dosing visits are dependent on the number of pre-surgical USL311 doses received and will occur within ± 3 days of scheduled visit.Surgery (re-resection) Visit:

For subjects randomized to pre-surgical treatment with USL311, Surgery (re-resection) may occur at any time from Day 2 onwards; USL311 will be administered daily until surgery. For subjects randomized to receive no USL311 treatment prior to surgery, Surgery (re-resection) will occur following Screening with the Screening Day -28 to Day -1 window calculated relative to day of surgery.

The following assessments or procedures will be performed during the day of Surgery (re-resection) for only those subjects randomized to no pre-surgical treatment:

- Assessment of inclusions/exclusion criteria (see Section 5.2)

- Physical examination (see Section 8.4.5.1)
- Neurological examination (see Section 8.4.5.2)
- Vital signs (see Section 8.4.6)
- Collect KPS (see Section 8.4.1)
- Holter-extracted ECG (see Section 8.4.7)
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
- Urine sample collection for UA (see Section 8.4.8)

The following assessments or procedures will be performed during the day of Surgery (re-resection) for all subjects in Part 4:

- Perform re-resection surgery (see Section 3.3.3)
- Collect any adverse events (see Section 9.1.1)
- Collect concomitant medication information (see Section 8.4.4)
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 11)
- CSF sample collection (see Section 8.6.2)
- Resected brain tissue sample collection (see Section 8.7.2)
- Tumor assessment by RANO criteria (see Section 8.2.3)
  - Performed ≤ 72 hours after surgery with EORTC QLQ-C30/BN20. If initiation of treatment following surgery is delayed for 3-4 weeks, a repeat baseline response assessment using the RANO criteria along with EORTC QLQ-C30/BN20 will be performed ≤ 7 days of study drug dosing.
- Perform EORTC QLQ-C30/BN20 (see Section 8.7.1)
  - Performed ≤ 72 hours after surgery with response assessment using RANO criteria. If initiation of treatment following surgery is delayed for 3-4 weeks, a repeat baseline EORTC QLQ-C30/BN20 along with response assessment using RANO criteria will be performed ≤ 7 days of study drug dosing.

See Table 6 for those randomized to pre-surgical treatment) and Table 7 (for those randomized to no pre-surgical treatment) for the schedule of events on the day of the surgery and timing of assessments for Part 4.

Cycle 1 visits:

Following surgery, all subjects (including those randomized to no pre-surgical treatment) will initiate treatment of USL311 in combination with lomustine. Cycle 1-Day 1 (post-surgery) will be initiated ≤ 28 days of Surgery, although treatment delays may be considered based upon discussions between Sponsor or designee and Investigator. Subjects must have fully recovered

from surgery, including surgical wound healing, prior to initiating therapy. Post-surgical Treatment with single agent USL311 in combination with lomustine will be administered in 42-day cycles.

The following assessments will be performed during Cycle 1:

- Assessment of inclusions/exclusion criteria (see Section 5.2)
  - Day 1 only
- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- USL311 administration in clinic (see Section 6.1.1.3)
  - Day 1, Day 2, Day 8, Day 15, Day 22, Day 29, and Day 36
- Lomustine administration (see Section 6.1.1.4)
  - Day 2 only
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Collect KPS (see Section 8.4.1)
  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1, Day 2, Day 8, Day 15
- Real-time bedside ECG (see Section 8.4.7)
  - Day 1 only
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Blood sample for cytochrome P450 genotyping (see Section 8.4.8.2)

  - Day 1 only
- Blood sample for optional pharmacogenomics analyses (see Section 8.4.8.3)
  - Day 1 or may be collected at a later visit if determined appropriate per the clinical site
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 1
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 1
- Blood sample collection for USL311 PK analysis (see Section 8.6.1; Table 12)
  - Day 1, Day 2, Day 8, and Day 15
- Blood sample collection for lomustine PK analysis (see Section 8.6.1; Table 12)
  - Day 2 only

The study visits for Part 4 Cycle 1 are on Day 1, Day 2, Day 8, Day 15, Day 22, Day 29, and Day 36. See Table 6 (for those randomized to pre-surgical treatment) and Table 7 (for those randomized to no pre-surgical treatment) for the schedule of events on each day of the cycle and timing of assessments. For Cycle 1-Day 1 and Day 2 visits will occur on consecutive days, whereas all remaining Cycle 1 visits will occur on the scheduled study day or within 3 days post the scheduled study day.

Cycle 2+ Visits:

The following assessments will be performed during Cycle 2 and all subsequent cycles:

- USL311 dispensing (see Section 6.6.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- USL311 administration (see Section 6.1.1.3)
  - Day 1, Day 2, Day 8, Day 15, Day 22, Day 29, and Day 36
- Lomustine administration (see Section 6.1.1.4)
  - Day 2 only
- Physical examination (see Section 8.4.5.1)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Neurological examination (see Section 8.4.5.2)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Vital signs (see Section 8.4.6)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Collect KPS (see Section 8.4.1)

  - Day 1 only
- Holter-extracted ECG (see Section 8.4.7)
  - Day 1 then if clinically indicated
- Real-time bedside ECG (see Section 8.4.7)
  - If clinically indicated
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
  - Day 1 only

- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
  - Day 1, Day 8, Day 15, Day 22, Day 29, and Day 36
- Urine sample collection for UA (see Section 8.4.8)
  - Day 1 only
- Tumor assessment by RANO criteria (see Section 8.2.3)
  - Cycle 2-Day 1, then every 6 weeks (± 7 days) thereafter and at 6 months (± 7 days)
- Adverse event monitoring (see Section 9.1.1)
  - Continuous through Cycle 2+
- Concomitant medication monitoring (see Section 8.4.4)
  - Continuous through Cycle 2+
- Perform EORTC QLQ-C30/BN20 (see Section 8.7.1)
  - Every 6 weeks (± 7 days) and at 6 months (± 7 days)

The study visits for Part 4 Cycle 2+ are on Day 1, Day 2 (only if lomustine is administered in the clinic), Day 8, Day 15, Day 22, Day 29, and Day 36. See Table 6 (for those randomized to pre-surgical treatment) and Table 7 (for those randomized to no pre-surgical treatment) for the schedule of events on each day of the cycle and timing of assessments. For all visits in Cycle 2+ (up to but not including the End of Treatment Visit), visits will occur within ± 3 days of scheduled visit.

7.3. End of Treatment/Follow-up Visit

The Treatment Phase will end and subjects will discontinue treatment if any of the following occurs:

- Subject experiences progressive disease, treatment failure or relapse
- Subject experiences unacceptable toxicity (see Section 6.1.4)
- Subject meets any of the withdrawal criteria (see Section 5.5)
- The study is terminated by the Sponsor

The End of Treatment Visit will occur ≤ 14 days of the decision to discontinue on-study treatment, whereas the Follow-up Visit will occur ≤ 28 days of the last dose of USL311. The End of Treatment visit may be the same as the Follow-up Visit; however, the Follow-up Visit should occur as close to 28 days after the last dose USL311 treatment as possible, per Investigator discretion. Treatment related AEs ongoing at End of Treatment Visit should be followed to resolution or until the Investigator considers them chronic or stable. The Follow-up Visit, or the End of Treatment Visit, as applicable, will be considered the last Treatment Phase visit.

The following assessments will be performed during the End of Treatment and Follow-up Phase:

End of Treatment Visit:

- Physical examination (see Section 8.4.5.1)
- Neurological examination (see Section 8.4.5.2)
- Vital signs (see Section 8.4.6)
- Collect KPS (see Section 8.4.1)
- Holter-extracted ECG (Parts 3 and 4 only; see Section 8.4.7)
- Safety ECG (Parts 1 and 2 only; see Section 8.4.7)
- Blood or urine sample collection for serum or urine pregnancy test, respectively, for all females of child bearing potential (see Section 8.4.9)
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
- Urine sample collection for UA (see Section 8.4.8)
- Tumor assessment by RECIST v.1.1 criteria (Parts 1 and 2 only; see Section 8.2.2) or RANO criteria (see Section 8.2.3)
- Collect any adverse events (see Section 9.1.1)
- Collect concomitant medication information (see Section 8.4.4)
- Perform EORTC QLQ-C30/BN20 (Parts 3 and 4 only; see Section 8.7.1)

Follow-up Visit:

- Physical examination (see Section 8.4.5.1)
- Neurological examination (see Section 8.4.5.2)
- Vital signs (see Section 8.4.6)
- Collect KPS (see Section 8.4.1)
- Blood sample collection for clinical laboratory tests (see Section 8.4.8)
- Collect any adverse events (see Section 9.1.1)
- Collect concomitant medication information (see Section 8.4.4)

See Table 1 – Table 7, for the schedule of events and timing of assessments for Parts 1a, 1b, 2, 3 (pre-surgical treatment), 3 (no pre-surgical treatment), 4 (pre-surgical treatment) and 4 (no pre-surgical treatment).

7.4. Long-term Follow-up Phase

All subjects who discontinue from study treatment, and are willing to be contacted, will be followed for survival information. The Long-term Follow-up Phase will begin after the Follow-

up Visit, or the End of Treatment Visit, as applicable. If a subject is unable to attend the End of Treatment Visit, the Long-term Follow-up Phase will begin upon investigator determination that the subject has ended the Treatment Phase. Survival follow-up information will be collected via telephone calls and/or clinic visits quarterly (every 3 months ± 1 week) until death, loss to follow-up, End of Study (refer to Section 3.4), withdrawal of consent, initiation of subsequent treatment for the target tumor, administrative reasons, or study termination by the Sponsor.

In Part 3 and Part 4, if a subject discontinues treatment for non-progression reasons and has not initiated any new oncology treatment, a 6 month visit for response assessment using RANO criteria and for collecting the EORTC QLQ-C30/BN20 health-related QOL questionnaire will occur.

7.5. Unscheduled Visits

As appropriate, assessments at any Unscheduled Visit should follow the assessment schedule at the closest weekly visit.

8. EFFICACY AND SAFETY ENDPOINTS AND ASSESSMENTS

The Study Schedules of Visits and Assessments in Table 1 – Table 7 describe the specific timing of required assessments and procedures for each part of the study. Refer to the specific table for each study part for this information.

8.1. Efficacy Endpoints

The efficacy endpoint of PFS-6m (secondary endpoint for Parts 1 and 2 and primary endpoint for Parts 3 and 4) will be determined in all subjects with solid tumors utilizing RECIST v.1.1 criteria and in subjects with primary brain tumors (e.g., GBM) utilizing RANO criteria. During Parts 1 and 2, in subjects with advanced solid tumors, tumor progression/response will be determined by local site reads and evaluations will be performed by qualified readers who have been trained in the applicable criteria. RECIST v.1.1 or RANO criteria will be used as applicable. In Parts 3 and 4, in subjects with relapsed/recurrent GBM, tumor progression/response will be based on blinded independent central review (BICR). RANO criteria will be used for all subjects. In Parts 3 and 4, site reads will be performed using RANO criteria by qualified readers who have been trained in the applicable criteria and the site reads will be performed primarily for subject management.

Secondary efficacy outcomes will also include ORR, DCR, PFS, and OS for all 4 study parts.

8.2. Efficacy Assessments

A secondary objective of Parts 1 and 2 of the study and primary objective of Parts 3 and 4 of the study is to make a preliminary assessment of the efficacy of USL311 in subjects with advanced solid tumors (Parts 1 and 2) or subjects with relapsed/recurrent GBM (Parts 3 and 4). Tumors will be assessed radiographically and response to treatment will be determined by RECIST v.1.1 or RANO criteria. RECIST v.1.1 criteria, will be utilized by trained and qualified site radiologist(s) in evaluating advanced solid tumor subjects in Parts 1 and 2. RANO criteria will be utilized by trained and qualified radiologist(s) in evaluating primary brain tumor subjects and GBM subjects in all 4 study parts.

Allowable imaging modalities for tumor measurements in Parts 1 and 2 include contrast-enhanced CT and contrast-enhanced MRI; contrast-enhanced computed tomography (CT)/positron emission tomography (PET) will only be accepted if the CT portion of the scan is performed with IV contrast enhancement and of diagnostic quality. Ultrasound, radiographs, or other imaging modalities may be utilized for standard-of-care management but cannot be used to select target or nontarget lesions at baseline or for tumor response evaluation. For the RANO criteria, contrast enhanced MRI is preferred. CT is an option ONLY for subjects unable to undergo MR imaging due to safety concerns. To ensure comparability, the baseline radiographs/scans and subsequent radiographs/scans to assess response should be performed using identical techniques, unless otherwise clinically required.

References for the RECIST v.1.1 criteria are included in Appendix 5 and for the RANO criteria in Appendix 6.

8.2.1. Evaluation of Response and Progression

Tumor response and progression can frequently be difficult to measure directly. Serial physical/neurological exams and CT/MRI scans may provide a guide to the actual course.

Detailed information about the evaluation of response and progression will be included in the Imaging Site Procedural Manual and Imaging Core Lab Manual.

8.2.2. Response Evaluation Criteria in Solid Tumors (RECIST)

The assessment of tumor response via RECIST v.1.1 criteria (56, 57) will be used for subjects with advanced solid tumors in Phase 1 (Parts 1 and 2). The article describing RECIST v.1.1 criteria is included in Appendix 5.

8.2.3. Response Assessment in Neuro-Oncology Criteria (RANO)

The assessment of tumor response via RANO criteria (58) will be used for subjects with primary brain tumors in Phase 1 (Parts 1 and 2) and subjects with GBM in Phase 2 (Parts 3 and 4). The article describing RANO criteria is included in Appendix 6, Safety Endpoints.

8.3. Safety Endpoints

The safety endpoints of interest are incidence of treatment emergent adverse events (TEAEs), including DLTs and SAEs; change in clinical laboratory tests (serum chemistry, hematology, coagulation assessments, UA); change in vital signs; change in ECGs (including real-time bedside ECG); change in physical examination; change in neurological examination; change in concomitant medication use; and change in BMP levels (Parts 1 and 2).

8.4. Safety and Screening Assessments

Safety in this study will be determined from evaluation of DLTs, (S)AEs, clinical laboratory assessments, ECGs, vital signs assessments, physical examinations, neurological examinations, concomitant medications and BMP levels (Parts 1 and 2). All subjects will be evaluable for safety.

SAEs will be recorded beginning at the time of signing of the ICF until the Follow-up Visit. If an SAE is received after the Follow-up Visit and is assessed by the Investigator as being at least possibly related to study drug(s), it will be reported to the Sponsor. All AEs ongoing at the End of Treatment Visit will be followed until resolution or until the condition is considered chronic/stable by the Investigator or the initiation of new anticancer therapy. The determination, evaluation, and reporting of AEs will be performed as outlined in Section 9.

The NCI CTCAE version 4.03 will be used to grade the severity of AEs in this study. Subjects will be assessed for AEs at each clinical visit and as necessary throughout the study.

8.4.1. Karnofsky Performance Status (KPS) Scale Criteria

Performance status will be measured using the KPS Scale (see Appendix 7).

The KPS Scale index allows subjects to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual subjects. The lower the KPS score, the worse the survival for most serious illnesses

(59). It is recommended, where possible, that a subject's performance status is assessed by the same individual throughout the study.

8.4.2. Medical History

Medical histories will be obtained according to the site's standard operating procedures, if applicable, or as determined by the Investigator or standard of practice. Medical histories will include demographic data (date of birth, sex, race, and ethnicity); histories of acute, chronic, or infectious disease; surgical histories; and any reported conditions affecting major body systems. All findings on medical history will be evaluated by the Investigator for clinical significance. Any new or clinically significant changes in the subject's medical history between signing of the IRB/IEC-approved ICF and the first dose of study drug(s) will be recorded on the AE eCRF page.

8.4.3. Oncologic History

Oncologic histories will be obtained according to the site's standard operating procedures, if applicable, or as determined by the Investigator or standard of practice. Oncology history will include disease course and prior cancer treatments including radiotherapy, surgery and systemic therapies. For systemic therapies only, the date of last therapy and best response to therapy should be documented for each therapy, if possible. Any residual toxicity related to prior treatments should be documented in the eCRF. All findings on oncologic history will be evaluated by the Investigator for clinical significance. Any new or clinically significant changes in the subject's oncologic history between signing of the IRB/IEC-approved ICF and the first dose of study drug(s) will be recorded on the AE eCRF page.

In Parts 3 and 4, the prognostic markers O6-methyl-guanyl-methyl-transferase (MGMT) promoter methylation status and isocitrate dehydrogenase 1 (IDH-1) mutational status should be documented in the eCRF if the status has already been determined as part of their clinical care.

8.4.4. Concomitant Medications

All concomitant medications (including over-the-counter and herbal treatments) will be recorded from 28 days before the first dose of study drug(s) until the Follow-up Visit. For primary brain tumor and GBM subjects, corticosteroid use including any changes in dose and/or frequency will be documented for RANO criteria assessment.

For the first dose of USL311 for each subject during Part 1 and Part 2, administration information for as needed (PRN) medications will be collected from 24 hours prior to USL311 administration through clinic discharge. The information collected will include the name of the medication, dose administered, and administration date and time and will be documented in the designated eCRF.

Any medication administered prior to the Follow-up Visit and with a stop date on or after the first dose of study drug(s) (or indicated as continuing) will be documented as concomitant medications. The reported medications will be reviewed and evaluated by the Investigator to determine if they affect a subject's eligibility or continued participation in the study.

8.4.5. Clinical Examinations

Any clinically significant abnormality identified during the physical or neurological examinations at Screening will be recorded in the subject's medical history. Any new clinically significant findings/abnormalities or worsening of Screening findings that meet the definition of an AE must be recorded as both an examination finding and as an AE.

8.4.5.1. Physical Examination

Physical examinations at Screening, Baseline (for Part 1 only) and Day 1 of Cycle 2+, and at the Follow-up visit will be complete assessments in Parts 1 and 2. Physical examinations at Screening, Day 1 of each cycle post-surgery, and at the Follow-up visit should be complete assessments in Parts 3 and 4. Other scheduled physical examinations for all study parts may be abbreviated, at the discretion of the Investigator, to identify changes from baseline or evaluate changes based on the subject's clinical symptoms. The complete physical examination will include assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, and extremities. Height, weight, body mass index (BMI), and BSA (calculated using DuBois and DuBois equation) will be recorded. Weight, BMI, and BSA to be reported at each visit with a scheduled physical examination, height at Screening Visit only.

8.4.5.2. Neurological Examination

Neurological examinations will be performed during all study parts. Neurological examinations will be performed according to the schedules shown in Table 1 – Table 7 in the Schedule of Visits and Assessments.

Neurological examinations at Screening and at Follow-up will be complete assessments. During the first day of USL311 dosing a complete neurological examination will be performed prior to dosing and more than 4 hours after oral dosing, prior to clinic discharge (for Part 1a, the complete neurological exam was to be performed anytime between the 5 hr nominal PK time point up to clinic discharge). During other USL311 dosing days for Cycle 1, neurological examinations will be performed prior to in-clinic dosing and may be abbreviated examinations. Other scheduled neurological examinations will be performed prior to dosing only, as applicable, and may be abbreviated examinations, at the discretion of the Investigator, to identify changes from baseline or evaluate changes based on the subject's clinical symptoms.

In Part 1a, neurological examinations will be performed ≤ 0.5 hr (mid-infusion) and ≤ 1 hr (end of infusion) after the nominal time point unless otherwise stated.The complete neurological exam will consist of evaluations of the following: Mental status (including orientation, memory and quality/fluency of speech), cranial nerves II-XII, motor strength of the upper and lower limbs, deep tendon reflexes, sensory exam, station and gait, Romberg test, coordination, nystagmus, and abnormal movements.

The abbreviated neurological examination will consist of the following: Mental status (including orientation, memory and quality/fluency of speech), finger-to-nose test, heel-to-shin test, rapid alternating movements, nystagmus, and tremor or other abnormal movements. In Part 1a, during the infusion of USL311, the abbreviated neurological exam may be performed in a manner to allow for minimal disruptions in dosing.

8.4.6. Vital Signs

Vital signs will be measured and managed according to the site's standard operating procedures, if applicable, or as determined by the Investigator or standard of practice. Vital signs will consist of blood pressure (systolic and diastolic blood pressure, mmHg), pulse rate (bpm), respiratory rate (breaths/min), and temperature (°C or °F). Subjects should be in a semi-recumbent position for at least 5 mins prior to the collection of vital signs. Vital signs at each nominal time point will be collected ± 10 mins relative to the nominal time point. When multiple assessments are scheduled at the same nominal time point ECG assessment (including semi-recument positioning, which is to begin at least 20 minutes prior to the nominal time point, will be completed first, and remaining assessments will be collected after the ECGs, with the PK sample being collected at the nominal time point.

Vital signs will be collected prior to in-clinic USL311 dosing and prior to clinic discharge. Additional vital signs assessments should be performed if clinically indicated.

Any clinically significant change in vital signs will be recorded as an AE. Clinically significant changes in vital signs should be confirmed by a repeat measurement (within 5 minutes of the original measurement) before recording as an AE.

8.4.7. Electrocardiogram Monitoring

Cardiovascular assessments will be completed with the use of Holter monitors provided by a central reader, utilizing three different approaches to collect and monitor cardiovascular safety: Holter-extracted ECGs, Safety 12-lead ECGs and real-time bedside ECG and heart rate monitoring.

The ECG variables will include, but are not limited to, ventricular heart rate/RR interval and the PR, QRS, QT, and QTc (QTcB and QTcF) intervals. The Investigator will review the ECG report and indicate the clinical significance of all abnormal findings, and then sign and store the ECG report in the subject's study file. Any clinically significant change in the ECG per the CTCAE criteria will be recorded as an AE.

For grading of QT prolongation according to CTCAE criteria, the Fridericia-corrected QT interval, QTcF, will be utilized.

To determine eligibility for initiating treatment, the last safety ECG performed prior to the first administration of study drug(s) will be used as baseline. The baseline QTcF interval must be ≤ 450 msec before the first dose of study drug(s). If the baseline QTcF interval is > 450 msec, the subject will not be eligible for the study. Prior to subsequent in-clinic dosing, subjects will be eligible to receive the dose of USL311 if the predose QTcF interval is < 501 msec, per Investigator discretion. If any post-infusion or post-dose QTcF interval is ≥ 501 msec, repeat ECG assessments will occur at least every 30 minutes until the QTcF interval is < 501 msec. USL311 dosing may be interrupted, per Investigator discretion, based on findings from real-time bedside ECG or scheduled and unscheduled ECG assessments.

Frequency of ECGs should be increased if/when clinically indicated and may be included at subsequent cycles if clinically indicated.

Holter-Extracted Electrocardiograms

12-Lead ECGs will be extracted from the Holter monitor by the ECG central reader and interpreted according to their standard operating procedures. The central reader will extract ECGs in triplicate with replicates at least 2 minutes apart. To facilitate the 12-lead ECG extractions, subjects will be required to rest with the Holter monitor in place in a semi-recumbent position during the length of the ECG collection period and for at least 5 minutes prior to the ECG collection period. Each ECG collection period spans from -15 minutes to -5 minutes prior to the nominal time point. Therefore, subjects should begin to rest in the semi-recumbent position at least 20 minutes prior to the ECG extraction nominal timepoint (e.g., if the nominal timepoint corresponds to 10:00 am, the subject should begin to rest in a semi-recumbent position no later than 9:40 am, so that ECG collection can take place between 9:45 and 9:55 am). Once available, the ECG reports for the extractions will be provided to the site by the central reader for assessment of clinical significance and AE reporting, as appropriate, but will not be available for real-time safety assessment. Instead, the scheduled safety ECGs are to be printed and used for real-time safety assessment.

Additionally, in Part 2, for the baseline ECG extractions on Cycle 1-Day 1, subjects should rest in the semi-recumbent position from -80 min (i.e., 20 minutes prior to the -60 minute nominal time point) through -20 min prior to lomustine administration.

During Part 1, time-matched ECGs will be used on Day -1 and Day 1 of Cycle 1. During Day -1, collection will occur for approximately 8.5 hours occurring at the equivalent time points (± 30 mins) as those planned on Day 1. The approximate clock times at which the ECG collections are planned to occur on Day 1 should be utilized for the corresponding time points on Day -1 for ECG collections. Timepoints for the ECG extractions for all study Parts are described in Table 1 – Table 4.

For DLT assessment based on extracted ECGs baseline is defined as the closest time-matched extracted ECG (triplicate average) from Cycle 1-Day -1 for Part 1 or the average of the pre-dose Cycle 1-Day 1 ECG extractions for Part 2.

Safety Electrocardiogram

During Part 1 and Part 2, safety ECGs will be generated as PDFs or printouts of ECGs from the provided laptop by clinic staff at scheduled timepoints during the visits, for the purpose of monitoring subject safety and treatment decisions by the Investigator. Safety ECGs at the Screening and End of Treatment visits will be recorded in triplicate with replicates at least 2 minutes apart. Single (i.e., non-triplicate) safety ECGs will also be collected at the timepoints described in Table 1 – Table 3.

Prior to performing safety ECGs, subjects will rest in the semi-recumbent position for at least 5 minutes and remain in this position throughout the ECG collection period. When safety ECGs are to be collected at the same nominal time as an ECG extraction, the safety ECGs should be collected during the ECG extraction period which spans from -15 minutes to -5 minutes prior to the nominal time point. Additional safety ECGs may be collected per Investigator discretion and should be collected for clinically significant changes from baseline. Frequency of safety ECGs should be increased if/when clinically indicated and may be included at subsequent cycles if clinically indicated. Safety ECG printouts from the equipment

may be collected per Investigator discretion and should be collected for clinically significant changes from baseline.

For DLT assessment based on safety ECGs, baseline is defined as the last ECG collected prior to the first dose of study drug.

Real-Time Bedside ECG and Heartrate Monitoring

Real-time bedside ECGs and heart rates will be monitored by clinic staff from prior to in-clinic dosing, or start of infusion with USL311 through clinic discharge (up to approximately 6 hours post or dosing) on days where real-time monitoring is required. Real-time monitoring of ECG and heart rate will be accomplished through the use of Holter monitors with real-time ECG waveform displays on a bedside laptop. Holter monitors will also be equipped with alerts for heart rates above a set threshold. Real-time monitoring will be performed at subsequent cycles as clinically indicated.

8.4.8. Clinical Laboratory Assessments

All routine clinical laboratory assessment will be performed by the study center's local laboratory facilities. Sampling for serum chemistry, hematology, UA and coagulation assessments can be drawn within 24 hours prior to study drug administration. Laboratory assessments scheduled for the day of study drug dosing must be available and assessed for toxicity before dosing. All clinical laboratory results must be available and reviewed by the Investigator or sub-Investigator prior to start of subsequent treatment cycles of study drug(s).

The total volume of blood loss for clinical safety laboratory assessments will be approximately 20 mL per clinic visit. Subject fasting is not required before collection of clinical laboratory blood or urine samples. All subjects will have the clinical laboratory tests performed as listed in Table 19. The last assessment collected prior to the Cycle 1-Day 1 dose will be considered the baseline.

Table 19: Clinical Laboratory Tests

Serum Chemistry[a]		
Albumin Alkaline Phosphate (ALP) Alanine Aminotransferase (ALT) Aspartate Aminotransferase (AST) Bicarbonate (CO_2) Blood Urea Nitrogen (BUN) (or urea as applicable)	Calcium Chloride Creatinine Creatine Phosphokinase (CPK) Gamma-glutamyltransferase (GGT) Glucose Lactate Dehydrogenase (LDH)	Magnesium Phosphorus or Phosphate Potassium Sodium Total Bilirubin Total Protein Uric Acid
Hematology		
Absolute Neutrophil Count (ANC) Absolute Lymphocyte Count (ALC)	Hematocrit (Hct) Hemoglobin (Hgb) Mean Corpuscular Volume (MCV) Platelet Count	Red Blood Cell (RBC) Count White Blood Cell (WBC) Count with Differential
Urinalysis[b]		

Blood Bilirubin Glucose	Ketones Leucocyte esterase Nitrite	pH Protein Specific gravity
Coagulation Assessments		
Activated Partial Thromboplastin Time (aPTT) / Partial Thromboplastin Time (PTT)	International Normalized Ratio (INR)	Prothrombin Time (PT)

[a] Baseline potassium, sodium, calcium (corrected for albumin) and magnesium levels below lower limit of normal must be corrected to within the normal range, or above the ULN if considered not clinically significant per the Investigator, prior to starting study drug.

[b] Dipstick UA; microscopy if clinically indicated. Potentially clinically significant findings include a positive test for protein (does not include trace findings), blood, nitrite and/or leukocyte esterase.

Blood and urine samples will be collected by qualified study center personnel and those samples to be used for clinical laboratory assessment will be sent to be analyzed by the local laboratory for the serum chemistry, hematology, UA, and coagulation assessments.

Note: A single urine sample may be collected and split for BMP biomarker, urine dipstick analysis, and/or urine pregnancy testing.

8.4.8.1. Abnormal Clinical Laboratory Findings

The Investigator will exercise medical judgment in deciding whether abnormal laboratory values are clinically significant. In some cases, significant changes within the range of normal will require similar judgment by the Investigator. In general, an abnormal laboratory value should be considered clinically significant if it results in treatment or prompts more intensive diagnostic evaluation. Repeating a laboratory test is not, in itself, a criterion for considering a result as clinically significant.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Clinical laboratory results should be compared to baseline values and only those values that have clinical sequelae will be considered clinically significant. Each clinically significant laboratory result should be recorded as an AE (see Section 9.1.3). The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE eCRF page.

8.4.8.2. Cytochrome P450 Genotyping

Blood samples for genotyping of CYP2D6 and CYP3A will be collected during all 4 study parts according to the site's standard procedures, if applicable, or as determined by the Investigator or standard of practice. The date and time of each collection should be recorded on the appropriate eCRF page. Further information for the handling, collection, storage, and shipping of genotyping samples will be provided in a separate laboratory sample handling

manual. Samples will be stored at a long-term storage facility under contract by the Sponsor and analyzed up to a maximum of 5 years after study completion, after which time the samples will be destroyed by incineration. Subjects have the right to refuse and to have their samples destroyed at any time. At the conclusion of the study, samples will be de-identified and handled in accordance with FDA guidance or other country specific guidances, as applicable.

8.4.8.3. Pharmacogenomics

In addition to the sample collected for cytochrome P450 genotyping, a sample may be collected during all study parts from subjects who have consented to participate in the pharmacogenomics component of the study. Participation is optional and a separate IRB/IEC approved informed consent form must be obtained for participation. Subjects who do not wish to participate in the pharmacogenomics research may still participate in the study. For those subjects that consent to participate in the pharmacogenomics component, an 8 mL blood sample will be collected for DNA isolation and pharmacogenomics analysis. In the event of DNA extraction failure, a replacement blood sample may be requested from the subject.

Data will be analysed in the context of this study but may also be explored in aggregate with data from other studies. Pharmacogenomics research may consist of analysis of one or more candidate genes or the analysis of genetic markers throughout the genome or analysis of the entire genome in relation to USL311. The pharmacogenomic samples will only be used for investigations related to the disease and the study treatment in the context of this clinical program. They will not be used for broad exploratory analyses for unspecified disease or population genetics. DNA samples may be analysed for genetic factors contributing to the subject's response to USL311 or other study treatment, in terms of PK, efficacy, tolerability, and safety. Such genetic factors may include genes for drug metabolizing enzymes, drug transport proteins, genes within the target pathway, or other genes believed to be related to study treatment response. Some genes currently insufficiently characterised or unknown may be understood to be important at the time of analysis. The samples may also be used for the development of diagnostic tests related to USL311 (or study treatments of this class). The results of pharmacogenomic analyses may be reported in a separate study summary.

Data arising from all biosamples will be subject to the country-specific confidentiality standards. Further information for the handling, collection, storage, and shipping of genotyping samples will be provided in a separate laboratory sample handling manual. Samples will be stored at a long-term storage facility under contract by the Sponsor and analyzed up to a maximum of 5 years after study completion, after which time the samples will be destroyed by incineration. Subjects have the right to refuse and to have their samples destroyed at any time. At the conclusion of the study, samples will be de-identified and handled in accordance with FDA guidance or other country specific guidance's, as applicable.

8.4.9. Pregnancy Testing

Female subjects of childbearing potential are required to have a serum pregnancy test for beta-human chorionic gonadotropin (β-hCG) at Screening. If positive, a confirmatory serum (required) pregnancy test will be performed. Female subjects with a positive pregnancy test and subsequent confirmation at Screening do not meet eligibility criteria for enrollment. If

treatment with study drug is stopped for ≥ 14 days, subject should have a negative pregnancy test prior to restarting study drug(s).

Females are considered not of childbearing potential if they meet any of the following criteria:

- Postmenopausal with > 1 year since last menses and:
  - If younger than 65 years old, with a follicle-stimulating hormone (FSH) > 40 mIU/mL
  - If ≥ 65 years old and not on hormone replacement therapy (HRT), with a FSH > 30 mIU/mL
  - If ≥ 65 years old and on HRT, the FSH requirement is not applicable. Postmenopausal females on HRT will be allowed if the treatment is stable for at least 6 months prior to dosing of study drug(s)
- Written medical documentation of being sterilized (e.g. hysterectomy, double oophorectomy, bilateral salpingectomy). **Note:** Tubal ligation is not considered a form of permanent sterilization.

Additional pregnancy testing (serum or urine) will occur at regular intervals during each study part.

Blood and urine samples will be collected by qualified study center personnel and those samples to be used for clinical laboratory assessment will be sent to be analyzed by the local laboratory for the pregnancy screens. Refer to Section 9.3.3 for details on the reporting procedures to follow in the event of pregnancy.

Note: A single urine sample may be collected and split for BMP biomarker, urine dipstick analysis, and/or urine pregnancy testing.

8.4.10. Archived Tumor Histology

During the Screening eligibility assessment for all 4 study parts, the availability of tumor block and/or slides (stained and/or unstained) used for diagnosis of the solid tumor (Parts 1 and 2) or GBM (Parts 3 and 4) must be confirmed for each subject. As soon as possible following the administration of the first dose of study drug(s), the tumor slides should be sent to the identified specialty lab.

8.5. Pharmacokinetic Endpoints

Blood samples will be collected for analysis of whole blood concentrations and plasma concentrations of USL311 and its metabolites as well as plasma concentrations of lomustine. Blood and plasma PK parameters will be calculated for USL311 and its metabolites, and plasma PK parameters will be calculated for lomustine and/or its metabolites. Non-compartmental and/or compartmental-based PK methods will be utilized and will include the following PK parameters as supported by the available data:

Table 20: Pharmacokinetic Parameters

Dose Number	Pharmacokinetic Parameters
First Dose	C_{max}, t_{max}, t_{last}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}tau}$, $AUC_{0\text{-}\infty}$, AUC % Extrapolated, *CL/F, λ_z, Terminal $t_{1/2}$
Subsequent Doses	C_{max}, t_{max}, C_{min}, t_{min}, C_{avg}, Fluctuation Index (FI), $AUC_{0\text{-}tau}$, *CL_{ss}/F, λ_z, Terminal $t_{1/2}$, *V_Z/F; Accumulation Ratio and AUC Ratio

* Calculated for USL311 and lomustine (parent drug) only

Additional PK parameters (e.g., area under the moment curve [AUMC], Mean Residence Time, metabolite:parent ratios, blood:plasma ratios) may be calculated as warranted.

CSF collection (see Section 8.6.2) is not required in Parts 1 and 2 but may occur as part of a Phase 1 sub-study at select sites and in a subset of subjects. In Parts 3 and 4, CSF collection will occur in all subjects during the surgical resection procedure. CSF samples will be used to evaluate the biodistribution of USL311 and its metabolites to the CNS and for PK/PD correlations.

8.6. Pharmacokinetic Assessments

8.6.1. Whole Blood and Plasma Sample Collection

Blood samples will be collected for the determination of whole blood and plasma concentrations and PK. Samples will be collected according to the schedule in Table 8 for Part 1a, Table 9 for Part 1b, Table 10 for Part 2, and Table 11 and Table 12 for Parts 3 and 4. Additionally, at the occurrence of an SAE or DLT, unscheduled blood samples for USL311 and lomustine (Parts 2 and 4 only) should be collected as soon as possible relative to the event. Blood samples will be collected in the arm contralateral to that containing the IV catheter for USL311 infusion via a saline flush catheter or by direct venipuncture. Samples will not be collected from the site of study drug infusion. Approximately 4 ml of blood will be collected at each time point for the analysis of whole blood (2 mL) and plasma (2 mL) USL311, M10 and 4-AP concentrations. Approximately 4 mL of blood will be collected at each time point for the analysis of plasma lomustine and/or its metabolites concentrations (Parts 2 and 4 only). Samples will be collected in blood collection tubes containing EDTA K_2 anticoagulant, inverted several times, and immediately be placed in an ice bath. The clock times of all blood draws will be recorded and reported for each subject. All tubes will be labeled with the subject identification number, protocol, and planned blood draw time point.

The total volume of blood collected for USL311 PK analysis is approximately 88 mL for Part 1a, approximately 76 mL for part 1b, approximately 108 mL for Part 2, and approximately 112 mL for Parts 3 and 4 (including Pre-surgical USL311 dosing). The total volume of blood collected for lomustine PK analysis is approximately 64 mL in Part 2 and approximately 32 mL in Part 4.

Blood samples for determination of the whole blood and/or plasma concentrations of study drug/metabolites will be analyzed at contract laboratories designated by the Sponsor. Samples will be analyzed using a validated bioanalytical method. Additional exploratory PK analyses such as metabolite profiling and analysis of USL311 concentrations in blood fractions (e.g.,

WBCs, erythrocytes) may be conducted using the backup samples. If conducted, this analysis will be performed independent of the clinical study and described in a separate analysis report.

As PK data become available, some samples may become optional, if warranted based on ongoing PK data analysis during the study conduct. Specific samples and the rationale for making them optional will be documented.

Detailed instructions for PK sample collection, processing, storage, and shipping will be provided by the analytical laboratory.

8.6.2. Cerebrospinal Fluid Sample Collection

Collection of CSF will be used to evaluate the biodistribution of USL311 to the CNS and PK/PD correlations.

In Parts 1 and 2, CSF sample collection is not required. However, if determined to be feasible and appropriate, CSF may be collected as part of a sub-study at select sites and in a subset of subjects. In the sub-study, CSF samples would be collected by lumbar puncture according to each site's institutional procedures to obtain approximately 1 mL of CSF from each subject. Subjects with lumbar puncture contraindications, as determined by the Investigator, would not be eligible for the potential collection of CSF during Parts 1 and 2.

In Parts 3 and 4, all subjects will have a CSF sample collected during the re-resection surgical procedure. The CSF sample will be collected from the operative field at the time of resection. Approximately 1 mL of CSF will be collected from each subject.

Detailed instructions for CSF sample collection, processing, storage, and shipping will be provided by the analytical laboratory.

8.7. Exploratory Assessments

The goal of exploratory, biomarker, PD analyses will be to potentially correlate measurable markers with prognosis, clinical results and/or safety observations and inform future development of USL311. Additionally, quality of life (QOL) assessments will allow for subject reported improvements.

Biomarkers not currently listed in the protocol, and relevant to the drug activity, might be added to the list of biomarkers to be assessed if they are determined as relevant based on publication of research made available during the time this study is ongoing. If possible, archiving of biomarker samples (including tumor tissue samples) is recommended to facilitate future biomarker testing. For those subjects who consent, some samples may be stored for future research. Samples will be stored for a maximum of 15 years at a long-term storage facility under contract by the Sponsor. At a maximum, the samples will be destroyed after 15 years by incineration. Subjects have the right to refuse and to have their samples destroyed at any time. At the conclusion of the study, samples will be de-identified and handled in accordance with FDA guidance or other country specific guidance's, as applicable.

8.7.1. Quality of Life Assessment

In Phase 2, Q OL and subject reported outcomes will be assessed using the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire,

supplemented with a brain-cancer module (EORTC QLQ-C30/BN20). The assessment of quality of life via EORTC QLQ-C30/BN20 is described in Appendix 8.

The QLQ-C30 is a 30-item self-report questionnaire that has patients rate the items on a 4-point scale, with 1 — "not at all" to 4 — "very much." The questionnaire measures several domains, including physical functioning, role functioning, emotional functioning, cognitive functioning, social functioning, fatigue, pain, nausea and vomiting, and several single items (dyspnea, insomnia, anorexia, constipation, diarrhea, and financial impact). The BN20 consists of 4 scales comprising multiple items (future uncertainty, visual disorder, motor dysfunction, communication deficit) and 7 single items (headache, seizures, drowsiness, hair loss, itching, difficulty with bladder control, and weakness of both legs) and is intended to be completed by patients with primary brain tumors. The combined instrument takes an average of 8 minutes to complete.

8.7.2. Resected Tissue Collection

During Parts 3 and 4, tissue will be collected during the re-resection surgery (see Section 3.3.3) from each consenting subject. Since the identification of biomarkers that correlate with disease activity and the efficacy or safety of treatment is rapidly evolving, some analyses might be determined after or instead of the below mentioned analyses.

Tumor samples will be used to measure biomarkers or changes in biomarkers related to the CXCR4 pathway, and/or study drug effects when possible. Exploratory (translational) studies on resected tissue may be performed to determine biodistribution of USL311 in the tumor and assess pharmacodynamic markers (pCXCR4 profile, autophagy markers [e.g., LC3, Beclin-1], markers of vasculogenesis [e.g., CD11b] and cancer stem cell survival and proliferation [e.g., nestin, CD133]). Genetic testing for the prognostic markers MGMT promoter methylation status and IDH-1 mutational status may also be performed.

Samples will be analyzed at a laboratory designated by the Sponsor. Tissue samples collected for assessment of the exploratory objectives will be analysed using appropriately qualified and/or validated assay methods. Detailed instructions for tissue sample collection, processing, storage, and shipping will be provided by the specialty laboratory.

8.7.3. Collection of Other Biomarkers

Other biomarkers will be collected during the study; these include but are not limited to the following for analysis of target engagement and DIPL.

Target Engagement Biomarkers:

All blood samples for target engagement biomarkers will be collected through a saline flush catheter or by direct venipuncture in the limb contralateral to that containing the IV catheter for drug infusion, if applicable. Samples will not be collected from the site of study drug infusion. The clock times of all blood draws will be recorded and reported for each subject. All tubes will be labeled with the subject identification number, protocol, and planned blood draw time point.

The following biomarkers will be monitored in Parts 1 and 2 as markers of CXCR4 target engagement:

- **Hematopoietic CD34+ cell count:** Approximately 6 mL of blood will be collected at each nominal time point to measure whole blood CD34+ count. Samples will be collected in blood collection tubes containing EDTA K_2 anticoagulant. Samples will be collected at the nominal time points described in Table 8, Table 9 and Table 10. The total volume of blood collected for CD34+ count in Part 1a is approximately 36 mL, in Part 1b approximately 30 mL, and in Part 2 is approximately 36 mL.
- **SDF-1:** For Part 1a only, approximately 2 mL of blood samples will be collected at each time point to measure plasma SDF-1. Samples will be collected in blood collection tubes containing EDTA K_2 anticoagulant. Samples will be collected at the time points described in Table 8. The total volume of blood collected for SDF-1 samples in Part 1a is approximately 12 mL.
- **WBC Count and Differential:** Approximately 2 mL of blood will be collected at each nominal time point to measure whole blood WBC count and differential. Samples will be collected in blood collection tubes containing EDTA K_2 anticoagulant. Samples will be collected at the nominal time points described in Table 8, Table 9 and Table 10. The total volume of blood collected for WBC count in Part 1a is approximately 40 mL, in Part 1b is approximately 102 mL and in Part 2 is approximately 132 mL.

Blood samples for determination of the target engagement biomarkers will be analyzed at specialty laboratories designated by the Sponsor. Detailed instructions for biomarker sample collection, storage, and shipping will be provided by the laboratory.

DIPL Biomarker:

The following biomarker will be monitored in Parts 1 and 2 as a marker of DIPL:

- di-docosahexaenoyl (22:6)-bis(monoacylglycerol) phosphate (BMP): Urine samples will be collected to measure BMP as urine is the most sensitive matrix for USL311-induced BMP changes in the animal studies. Samples will be collected according to Table 1 – Table 3.

Urine samples for determination of BMP will be analyzed at a specialty laboratory designated by the Sponsor. Detailed instructions for biomarker sample collection, storage, and shipping will be provided by the specialty laboratory.

Note: A single urine sample may be collected and split for BMP biomarker, urine dipstick analysis, and/or urine pregnancy testing.

8.8. Appropriateness of Measures

Tumor response, based on radiological measurement and RECIST v.1.1 criteria (56, 57), is a standard and well-accepted efficacy endpoint in clinical oncology for solid tumors. Additionally, tumor response, based on radiological measurement and RANO criteria (58), is standard and well-accepted efficacy endpoint in clinical neuro-oncology.

The safety and PK endpoints in this study are standard and well-understood endpoints in clinical oncology.

For drugs targeting GBM, blood brain barrier penetration is critical and blood and plasma concentrations may not reflect concentrations within the CNS. CSF samples provide a direct and accessible measure of blood brain barrier penetration and CNS exposure. Moreover, nonclinical data indicates that CSF concentrations provide a reasonable surrogate of unbound drug concentrations in the CNS.

The assessment of QOL via EORTC QLQ-C30/BN20 is a well-accepted tool for the assessment of QOL. Health-related QOL, symptom burden, and neurocognitive measures have been validated in the brain tumor population as additional indicators of treatment benefit (60).

The PD assessments in blood samples (CD34+ cell count, WBC count and differential) were selected as systemic biomarkers of CXCR4 pathway modulation. CD34 is an antigen found on hematopoietic stem cells. Other agents that target the CXCR4 pathway (i.e, plerixafor) have been shown to mobilize CD34+ stem cells. USL311 has been demonstrated to mobilize WBCs in nonclinical studies (see Section 1.2.2.1) and clinical trials have demonstrated that other CXCR4 pathway modulators increase WBC count (61, 62).

Tissue sample biomarkers were selected for the following reasons and these include; target activity (pCXCR4 profile), autophagy markers (e.g., LC3, Beclin-1), markers of vasculogenesis (e.g., CD11b) and cancer stem cell survival and proliferation (e.g., nestin, CD133).

Nonclinical toxicology testing demonstrated that USL311 has the potential to cause DIPL. The BMP biomarker is a s ensitive and specific measure of DIPL (63). BMP is a lysosomal phospholipid that is increased in the tissues and biological matrices (urine, plasma, serum) of humans with DIPL. BMP that has been cleared or released by the kidney itself can be detected in the urine and urine sediment (64). The total volume of blood collected for study assessments will vary with each subject throughout the study and will depend on the duration of participation in the study. The total volume of blood collected during the study for any one subject is not expected to cause any harm.

9. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The Investigator or designee and research site staff are responsible for the detection, documentation, classification, reporting, and follow-up of events meeting the definition of anAE or SAE.

Spontaneously reported or observed AEs will be recorded throughout the study from the time of signing the ICF until the Follow-up Visit. AEs will be elicited using a non-leading question. Regardless of seriousness, intensity, or presumed relationship to study drug, all AEs will be recorded in the source documentation and entered on the appropriate eCRF from the time the subject signs the informed consent until the end of the Follow-up Visit (≤ 28 days after the last dose of study drug). After this time period, the only SAEs that need to be collected and reported to the Sponsor are those that are felt by the Investigator to be at least possibly related to study drug. All measures required for management of AEs will be recorded in the source documentation.

This study will utilize the NCI CTCAE version 4.03 for AE reporting.

9.1. Definitions

9.1.1. Adverse Events

An AE is any untoward medical occurrence in a subject administered a pharmaceutical product and may not necessarily have a causal relationship with the administered treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory abnormality, for example), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug. During the study, an AE can also occur outside the time that the study drug(s) was given (e.g., during a washout period).

Examples of AEs include the following:

- A symptom or disease not previously observed in the subject that emerges during the course of the study
- Exacerbation of a pre-existing illness following the start of the study
- Increase in frequency, or intensity of a pre-existing episodic event or condition
- Condition that leads to a medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion) may be an AE, but the procedure itself is not an AE
- Complications that occur as a result of non-drug protocol-imposed interventions (e.g., AEs related to Screening procedures, medication washout, or no treatment run-in)

An AE does not include:

- Relapse or progression of the underlying malignant disease; however, the associated signs, symptoms, or diagnosis should be recorded as AEs (e.g., “jaundice” due to new of increasing liver metastases, or “tumor pain” or “bone pain” due to progressive disease)

- Death due to the underlying malignant disease
- Day to day fluctuations of pre-existing disease or conditions present or detected at the start of the study that do not represent a worsening of the disease or condition
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalizations for cosmetic elective surgery; medical or surgical procedures such as endoscopy, or transfusion; social and/or convenience admissions)
- Overdose of either study drug or concurrent medication without any signs or symptoms

The Investigator will evaluate AEs using the following guidelines:

- Description of Event (if the event consists of a cluster of signs and symptoms, a diagnosis should be recorded [e.g., flu syndrome] rather than each sign and symptom)
- Onset Date
- Stop Date
- Intensity
- Seriousness
- Relationship to study drug
- Frequency
- Outcome
- Action taken (with regard to study drug): no change, permanent discontinuation, temporarily stopped)

9.1.2. Serious Adverse Events and Serious Unexpected Adverse Events

It is important to distinguish between SAEs and severe AEs. Severity is a measure of intensity, whereas seriousness is defined by the criteria provided below. An AE of severe intensity need not necessarily be considered serious. For example, a migraine headache that incapacitates a subject for many hours may be considered to be a severe AE, but it is not an SAE.

Seriousness, as provided in FDA Title 21, CFR Part 312 and the guidelines of the ICH GCP (CPMP/ICH/135/95), define an SAE as any untoward medical occurrence that occurs at any dose of study drug(s):

- Results in death,
- Is life-threatening (immediate risk of death at the time of the event),
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A serious and unexpected AE is an SAE that is not identified in nature, intensity, or frequency in the risk information set out in the Investigator's Brochure and/or on the label of the drug.

The Investigator must record whether or not the AE meets the definition of serious. If the event is serious, the investigator must complete an electronic SAE Report Form.

9.1.3. Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal laboratory findings (e.g., from clinical chemistry, hematology, or UA) or other abnormal assessments (e.g., from vital signs or ECG) should not be listed on the AE eCRF page unless signs or symptoms are present or the laboratory finding or assessment is deemed clinically significant by the Investigator or designee (confirmed by repeat laboratory testing). If a laboratory value or assessment is related to a medically defined new or worsening of a preexisting diagnosis or syndrome, the diagnosis or syndrome, not the individual laboratory values, will be recorded on the AE eCRF page. If a medically defined diagnosis or syndrome cannot be made and the subject is asymptomatic, a clinically significant laboratory or assessment value will be recorded as an AE.

All clinically significant abnormal laboratory results or assessments will be followed until they resolve (return to normal or baseline values) or stabilize, or until they are judged by the Investigator to be no longer clinically significant.

9.2. Evaluation of Adverse Events and Serious Adverse Events

The Investigator or designee is responsible for making an assessment as to the seriousness, intensity, causality, and outcome of an AE.

9.2.1. Classification of Adverse Event Intensity

The Investigator will determine the severity of each event by using NCI CTCAE v.4.03 grading criteria. Adverse events that do not have a corresponding NCI CTCAE term will be assessed according to the general guidelines for grading used in the NCI CTCAE as stated in Table 21 below.

If there is insufficient information to determine intensity, the AE must still be reported; the intensity/grading can be added after additional information is received. Classification for asymptomatic abnormalities will be according to the Investigator or designee's discretion and any applicable site standard operating procedures.

Table 21: Classifications for Adverse Event Intensity/Grading

Description	Grade
Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	1
Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	2
Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	3
Life threatening or debilitating: consequences; urgent intervention indicated	4
Death related to AE	5

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.
[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

9.2.2. Classification of Adverse Event Causality

For each recorded AE or SAE, the Investigator or designee must make an assessment of causality for USL311 (and for lomustine when applicable) based on the following criteria to determine the relationship between the AE or SAE and study drug(s). The Investigator must make a causality assessment (relationship to study drug[s]) based on the following 5 causality terms in Table 22.

Table 22: Relationship to Study Drug

Relationship	Attribution	Description
Unrelated to study drug	Not related	The AE is not temporally correlated with study drug(s) administration; the AE is clearly explained by another mechanism.
	Probably not related	The AE is not reasonably, temporally correlated with study drug(s) administration; is more likely explained by another mechanism.
Related to study drug	Possibly related	The AE is temporally correlated with study drug(s) administration; can be explained equally well by study drug(s) exposure or another mechanism.
	Probably related	The AE is temporally correlated with study drug(s) administration and is more likely explained by study drug(s) exposure than by another mechanism.
	Related	The AE is temporally correlated with study drug(s) administration; the AE is more clearly explained by study drug(s) exposure than by another mechanism.

9.2.3. Classification of Adverse Event Outcome

For each recorded AE or SAE, the Investigator or designee must make an assessment of outcome at the time of last observation. The outcome of AEs or SAEs will be documented as follows:

Table 23: Classifications for Adverse Event Outcomes

Classification	Definition
Fatal	The AE results in death.
Resolved	The AE or SAE has ended.
Improved	The AE or SAE is not yet resolved, but improving.
Resolved with Sequelae	The AE or SAE has ended but changes are noted from baseline that are stable or chronic.
Unresolved	The AE has not ended. An AE outcome can only be categorized as unresolved, if the AE is: *ongoing* at the end of the reporting period (i.e., after the Follow-up Visit) and the Investigator deems that further follow-up is not medically required; *lost to follow-up* after repeated unsuccessful attempts to contact the subject; *ongoing and referred* to the subject's physician or a specialist.

The Investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the causality assessment for individual AE/SAE reports, the Sponsor will promptly evaluate all reported safety information against cumulative product experience to identify and expeditiously communicate possible new safety findings to Investigators and applicable regulatory authorities.

9.3. Reporting Procedures

The reporting period for both AEs and SAEs is the period from the time signing the ICF through the Follow-up Visit (≤ 28 days after the last dose of study drug[s]). If an AE or SAE remains unresolved at the conclusion of the study, the Investigator will assess whether continued follow-up of the AE or SAE is warranted, and the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic. The Investigator should follow all unresolved AEs and SAEs until the events are resolved or stabilized, or the subject is lost to follow-up. Outcome of AEs (with dates) should be documented on the appropriate eCRF page(s) and in the subject's medical record.

9.3.1. Serious Adverse Events and Serious Unexpected Adverse Events

Any SAE, whether expected or unexpected, irrespective of relationship to study treatments, experienced by a study subject, will be reported to the Sponsor by the Investigator or designee, on an electronic SAE Report Form within 24 hours but no later than one business day of learning of the event. Information regarding the SAE will be transmitted to Chiltern International pharmacovigilance (PV); contact information and timelines are listed below in Table 24.

Table 24: SAE Reporting Information

Type of SAE	Reporting Timeframe	Primary Reporting Method	Primary Contact	Secondary Contact
Fatal or Life-threatening SAEs	Immediately from becoming aware of the SAE	Phone call to Proximagen and/or Chiltern Medical Monitors	Chiltern International PV Medical Monitor Phone: 423-990-0289 Email: global.SAEinbox@chiltern.com	Tze-Chiang Meng, MD Office: 952-658-7440 Mobile: 952-818-5850 Email: TCMeng@proximagen.com
	Immediately but no later than 24 hours from becoming aware of the SAE	Electronic SAE report form in EDC Merge Email	Chiltern International PV Email: global.SAEinbox@chiltern.com	Email: drugsafetyUSL311@proximagen.com
Non-Fatal or Life-threatening SAEs	Immediately but no later than 24 hours from becoming aware of the SAE.	Electronic SAE report form in EDC Merge Email	Chiltern International PV Email: global.SAEinbox@chiltern.com	Email: drugsafetyUSL311@proximagen.com

The Sponsor assumes responsibility for appropriate reporting of SAEs to the regulatory authorities. Reporting of SAEs to the appropriate regulatory authorities will be completed by the Sponsor or its representatives. The Sponsor, or its representatives, will also report to the Investigator, all SAEs (including those from other USL311 studies), that are unlisted and associated with the use of the study drug(s). The Investigator (or the Sponsor, where required) must report these events to the appropriate IRB)/IEC that approved the protocol (unless otherwise required and documented by the IRB/IEC).

All additional follow-up evaluations for SAEs will be reported to the Sponsor in the same timeframes as listed above. All SAEs must be followed until they are resolved (return to normal or baseline) or the subject's condition has stabilized, or until they are judged by the Investigator to be no longer clinically significant. If a subject dies, a death certificate and any available post mortem findings (including histopathology) must be provided to the Sponsor (or its designee).

9.3.2. Any Adverse Event

Regardless of seriousness, intensity, or presumed relationship to study drug(s), all AEs will be recorded in the source documentation and on the AE eCRF page. SAEs also require completion of the electronic Serious Adverse Event Form. If necessary, paper forms will be used as back up. Whenever possible, diagnoses will be recorded when signs and symptoms are due to a common etiology. In addition, the Investigator must record his or her opinion as to the intensity of the AE and whether the AE is related to study drug. All measures required for management of the AE will be recorded in the source documentation.

9.3.3. Pregnancy

Any subject who becomes pregnant during the study will be immediately withdrawn and will be scheduled for the End of Treatment and Follow-up Visits. Research site staff will report the pregnancy to Chiltern within 24 hours of learning of the pregnancy. The Investigator must complete the Pregnancy Notification Form provided by the Sponsor. The Investigator willperiodically follow up with the subject until delivery or termination of the pregnancy, providing necessary updated information to Chiltern using the Pregnancy Outcome Form. Information on the status of the mother and the child will be forwarded to Chiltern using the Pregnancy Notification Follow-up Form. Generally, follow-up will occur within 6–8 weeks following the expected delivery date. Any premature termination of the pregnancy will also be reported on this form.

Although pregnancy occurring in a clinical study is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons, will be recorded as an AE or SAE and will be followed as such. A spontaneous abortion is always considered to an SAE and requires completion of an SAE Form.

9.4. Follow-up of Adverse Events and Serious Adverse Events

All AEs and SAEs must be followed until they are resolved (return to normal or baseline), the subject's condition has stabilized, or until they are judged by the Investigator to be no longer clinically significant. Supplemental measurements and/or evaluations may be necessary to fully investigate the nature and/or causality of an AE or SAE. This may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. If the subject dies, a death certificate and any available postmortem findings (including histopathology) must be provided to the Sponsor.

If it becomes unlikely that any additional information can be obtained (e.g., subject or health care practitioner refuses to provide additional information, the subject is lost to follow-up), the Investigator or designee will ensure that the follow-up includes any pertinent supplemental investigations (e.g., laboratory tests or investigations, histopathological examinations or consultation with other health care professionals) to elucidate the nature and/or causality of the AE or SAE.

SAEs that occur after the Follow-up Visit will be collected only if the events are considered by the Investigator to be at least possibly related to study drug.

9.5. Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new

events and the measures taken and shall ensure that the IRB/IEC is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the Follow-up Visit. Investigators are required to report any events that may require the implementation of urgent safety measures to Sponsor within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- An immediate need to revise the study drug administration (i.e., modified dose amount or frequency not defined in protocol)
- Detrimental study conduct or management
- Discovery that the quality or safety of the study drug does not meet established safety requirements

10. DATA ANALYSIS AND STATISTICAL PROCEDURES

10.1. General Statistical Considerations

Complete details of the statistical analyses to be performed will be documented in statistical analysis plan(s), which will be completed prior to locking the database. This document will include more detail of analysis populations, summary strategies, and any amendments to the proposed analyses listed here, if necessary. Any deviations to the procedures outlined in the statistical analysis plan(s) will be outlined in the final study report. Additional statistical analyses other than those described in this section may be performed if deemed appropriate and will be described in the statistical analysis plan(s) (SAP).

Efficacy, safety, PK, PD and QOL assessments will be summarized separately for Phase 1 (Parts 1 and 2, the "dose-escalation cohorts") and Phase 2 (Parts 3 and 4, the "dose-expansion cohorts") of the study. Additional summaries and analyses of pooled Phase 1 and 2 data may also be generated. Part 1a (USL311 IV administration) may be analyzed separately; furthermore, subjects administered 2-hour and 4-hour infusions may be displayed separately. Details of the handling of Part 1a will be described in the SAP. Descriptive and summary statistics will be presented by dose group and overall, as appropriate. Statistics will include number of observations, mean, standard deviation, median, range, and inter-quartile range for continuous variables, and the number and percent for categorical variables; 95% or 90% confidence intervals will be presented where appropriate. Additional statistics such as geometric mean and coefficient of variation (CV%) may be calculated as warranted.

10.2. Interim Analysis

No formal interim analysis will be performed in this study. After each part in Phase 1, the MTD for single agent USL311 (Part 1) and USL311 in combination with lomustine (Part 2) will be determined by the DEC. The DEC will determine the RP2D which will be used for Phase 2 dosing for single agent USL311 (Part 3) and USL311 in combination with lomustine (Part 4).

10.3. Missing Data

Handling of missing data, drop-outs, outliers and censoring rules will be described in the SAP(s).

10.4. Analysis Data Sets

The following data sets will be defined for data analysis:

- **Full Analysis Data Sets** will include all subjects who receive at least one dose of USL311 or lomustine. This will be the analysis set for all efficacy analyses.
- **Safety Analysis Data Sets** will include all subjects who received at least one dose USL311 or lomustine and have at least one post-baseline safety evaluation. This will be the analysis set for all Safety analyses.
- **MTD-Evaluable Data Sets** will include all subjects in Phase 1 (dose-escalation) who complete one cycle of treatment and assessment or who discontinue before

completing the first cycle because of toxicity. This will be the analysis set for MTD determination in the Phase 1.

- **Pharmacokinetic (PK) Analysis Data Sets** will include all subjects who have received at least one dose of USL311 or lomustine and have at least one quantifiable post-dose blood or plasma concentration of either drug.
- **Pharmacodynamic (PD) Analysis Data Sets** will include all subjects who have received at least one dose of USL311 or lomustine and have at least one evaluable post treatment assessment for the given PD measure.
- **Response-Evaluable Analysis Data Sets** will consist of those subjects who have received at least one dose of USL311 or lomustine and have evaluable disease at baseline.
  - In Parts 3 and 4, subjects must have received at least one dose of USL311 or lomustine post-surgery and have at least one non-missing efficacy assessment (i.e., imaging or survival assessment)
- **Exposure-Response Analysis Data Sets** will be constructed for each PD or response endpoint to be evaluated for correlation with PK data. The exposure-response analysis sets will consist of all subjects in the PK Analysis Set that also have an evaluable PD/response pretreatment assessment and at least one post treatment PD/response assessment.

10.5. Dispositions, Demographics, and Other Baseline Characteristics

Demographics and baseline characteristics (e.g., age, sex, race, ethnicity, body weight) will be summarized using descriptive statistics for the Safety Population.

The number of subjects in each of the treatment groups will be presented, in addition to the number of subjects who complete each visit. The reasons for all post-randomization discontinuations will be tabulated and grouped by treatment and major reason. All deviations related to study inclusion or exclusion criteria, conduct of the trial, subject management, or subject assessment will be described.

10.6. Medical and Surgical History

Medical and surgical history will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA®) and the incidences will be tabulated for the Safety Population.

10.7. Efficacy Endpoints Analysis

Efficacy will be evaluated in both Phase 1 and Phase 2 components. In Phase 1, preliminary efficacy parameters such as PFS-6m, ORR%, DCR and OS will be determined using RECIST v.1.1 or RANO criteria as appropriate. The Phase 2 analyses will characterize efficacy in subjects in the dose expansion cohorts treated at the RP2D for USL311 as a single agent and in combination with lomustine as determined by % PFS-6m, PFS, OS, ORR%, DCR, and as defined by RANO criteria.

There is no formal hypothesis testing in this trial for efficacy endpoints. Approximately 20 evaluable subjects will be studied in each of the two Phase 2 dose-expansion groups to provide a preliminary estimate of efficacy in relapsed/recurrent GBM. The primary objective, PFS-6m, will be calculated with two-sided 90% confidence interval (CI) using K-M product-limit estimate of PFS. This will be performed based on both the full analysis set and response-evaluable set. Median PFS will be calculated using K-M product-limit estimates and presented with two-sided 90% CIs.

Every effort will be made to ensure complete, accurate and timely data collection, and to avoid missing data.

10.8. Safety Endpoints Analysis

Adverse event (AE) data will be descriptively evaluated. Descriptive statistics (e.g., number of observations, means, standard deviations, medians, maximum and minimum values) will be used to summarize continuous variables. Frequencies, proportions, and the exact 95% confidence intervals (CI), when appropriate, will be used to summarize categorical variables. Subject listings will also be provided.

In both Phase 1 and Phase 2, the incidence and duration of toxicities will be tabulated according to the NCI-CTCAE version 4.03. All laboratory results and vital sign measurements will be summarized using appropriate descriptive statistics. The schedule of assessments tables describes the timing of required evaluations.

Safety and tolerability will be evaluated in both Phase 1 and Phase 2 components. The Phase 1 primary analyses will include determination of the MTD and RP2D for USL311 as a single agent and in combination with lomustine. All available safety, tolerability, PK and PD data will be considered by Sponsor in dose escalation decisions.

Additional safety analyses other than those described in this section may be performed if deemed appropriate and will be described in detail in a separate analysis plan.

10.9. Pharmacokinetic Endpoints Analyses

Blood (as appropriate) and plasma concentrations of each analyte (USL311, M10, 4-AP, as well as lomustine and/or its metabolites) will be listed for each individual subject and descriptive statistics will presented for each time point by dose and treatment. As warranted, PK data will be presented in tables and graphical displays with time “0” assigned to the dosing time of each treatment.

Blood and plasma PK parameters for each analyte will be calculated using non-compartmental and/or compartmental methods (such as population PK analysis) as appropriate using actual sample collection times post-dose. Individual subject PK parameters will be listed and descriptive statistics will be calculated by dose and treatment. Mean and individual subject blood (USL311, M10 and 4-AP only) and plasma concentration-time profiles will be presented on rectilinear and semi-logarithmic scales stratified by dose and treatment.

Concentrations of USL311 and its metabolites in CSF and tumor samples will be listed for each individual subject and descriptive statistics will be calculated by dose and treatment.

Assessments of USL311 dose proportionality will be performed using standard methods (i.e., power model approach) and will be supported by graphical analysis.

Assessment of a PK drug-drug interaction between USL311 and lomustine will be via comparison of USL311 and lomustine PK parameters (AUC, C_{max}) when administered in combination to the PK parameters of each agent when administered alone. Statistical analysis will be performed using standard methods (i.e., analysis of variance [ANOVA], non-linear mixed effect modeling) and will be supported by graphical analysis.

Correlations between PK parameters and safety, efficacy and/or PD metrics may be explored graphically and if relationships are observed, exploratory analyses may be performed. Additional exploratory analyses may be conducted and will be described in detail in a separate analysis plan.

10.10. Pharmacodynamic Endpoints Analyses

PD data obtained from the exploratory/translational analyses will be summarized using descriptive statistics as appropriate. Correlations between PD measures and safety and/or efficacy metrics may be explored graphically and if relationships are observed, exploratory analyses may be performed. PD parameters (i.e., peak concentrations, area under the curve) may be calculated and additional exploratory analyses may be conducted and will be described in detail in a separate analysis plan.

10.11. Quality of Life/ Patient Reported Outcome Measures

In Phase 2, changes in health-related QOL and patient reported outcome measures will be assessed based on the EORTC QLQ-C30/BN20. The QLQ-C30 is a 30-item self-report questionnaire that measures several domains, including physical functioning, role functioning, emotional functioning, cognitive functioning, social functioning, fatigue, pain, nausea and vomiting, and several single items. The BN20 consists of 4 scales comprising multiple items (future uncertainty, visual disorder, motor dysfunction, communication deficit) and 7 single items (headache, seizures, drowsiness, hair loss, itching, difficulty with bladder control, and weakness of both legs).

10.12. Sample Size Calculation

There is no formal hypothesis testing in this trial, thus no calculation of sample size was calculated. See Section 5.1 for an approximation of the number of subjects to be enrolled in the study.

10.13. Data Collection and Electronic Data Capture

The study site will use a validated electronic data capture (EDC) system to enter subject data onto eCRFs. Data will be collected using the EDC system and entered into a quality controlled clinical database. Prior to the commencement of the study, items to be included in the clinical database will be determined and suitable paper source documents will be created to ensure the appropriate collection of all required data. Clinical staff conducting the study will record the required data onto source documents and will enter the appropriate data from source documents into the clinical database. All entries, corrections, and alterations will be made by the

Investigator or other authorized study personnel, and all data entries will be verified for accuracy and correctness. The EDC system is optimized for manual keying and review (including review by independent monitors) and maintains a full audit trail.

The study file and all source data will be retained at the clinical site until notification is given by the Sponsor for destruction.

11. ADMINISTRATIVE PROCEDURES

11.1. Regulatory Approval

This study requires application to the appropriate regulatory body in the countries concerned. The study will only be undertaken following receipt of written approval or acknowledgement of receipt (depending on local regulation) from the regulatory bodies by the Sponsor, or following submission to appropriate authorities, whichever is required by the respective countries.

This study requires authorization by any member state Regulatory Authority where the clinical study will be conducted in accordance with National law requirements. The study will only be undertaken by the Sponsor following receipt of written approval from the Regulatory Authority.

11.2. Institutional Review Board (IRB) or Independent Ethics Committee (IEC) Approval

The Sponsor or its designee must receive signed and dated written confirmation that the study protocol and ICF and assent forms have been approved or favorably reviewed by the IRB/IEC before the study center will be initiated. The IRB/IEC Membership Roster (or assurance number, if applicable) must also be supplied to the Sponsor or its designee before center initiation.

The Investigator will provide the IRB/IEC with all appropriate material, including the protocol, Investigator's Brochure (IB), the ICF including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and study drug supplies will not be shipped to the site until appropriate documents from the IRB/IEC confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at Sponsor or its designee. The approval document should refer to the study by protocol title and Sponsor protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. Sponsor will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC and Sponsor by the Investigator in accordance with applicable guidance documents and governmental regulations.

Any amendment(s) to the study protocol that affect the study design, study procedures, or risk to study subjects, and any corresponding change to the informed consent or assent forms, must be approved by the IRB/IEC before the change is implemented, in conformance with GCP. If any such changes are made to the informed consent or assent forms, subjects that are still active in the study will be re-consented using the new form(s).

11.3. Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and subject participation continued as long as the Investigators conducting the study do not find the hazards to outweigh the potential benefits.

Each subject will provide written, informed consent before any study-specific tests or evaluations are performed.

11.4. Study Personnel

The Investigator should maintain a list of appropriately qualified persons who are delegated to perform significant study-related duties. In addition, the Investigator should maintain a signature sheet to document signatures, initials, and study responsibilities of all persons authorized to make entries and/or corrections to the eCRF.

Prior to beginning the study, the Investigator at each site must provide to Sponsor or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators and research coordinators with significant study-related duties must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered and monitored by employees or representatives of the Sponsor. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. Sponsor's Regulatory Affairs Department, in conjunction with Sponsor Pharmacovigilance (or designee) will be responsible for the timely reporting of SAEs to appropriate regulatory authorities as required.

Clinical laboratory evaluations will be performed at the local laboratories associated with the study sites. The specific laboratories and instructions for sample collection, processing and shipment is provided in the laboratory manual. Special laboratory assessments (including, but not limited to, tumor tissue sample, WBC, CD34+ and BMP) will be performed at contract laboratories designated by the Sponsor.

11.5. Ongoing Information for Institutional Review Board

Unless otherwise instructed by the IRB/IEC, the Investigator (or his/her designee) must submit to the IRB/IEC at a minimum:

- Information on SAEs from the Investigator's study center, as soon as possible
- Expedited safety reports from the Sponsor or its representatives, as soon as possible
- Periodic or annual reports on the progress of the study

11.6. Data Handling and Record Keeping

11.6.1. Case Report Forms

The Investigator is responsible for the quality of the data recorded for this study. These recorded data should be a complete and accurate account of each subject's record collected during the study. Subject data that are collected may be substantiated by 2 types of source documents at the study center, paper and electronic (electronic source data is defined as electronic information not directly entered into the EDC system). Source data collected electronically or via paper will be entered onto the eCRFs in the EDC system. The eCRFs will be completed according to guidelines provided by the Sponsor or its designee.

Access to the EDC system will be granted to trained and authorized study personnel only, and user IDs and passwords must not be shared with other individuals. Only staff designated by the principal Investigator on the Delegation of Authority form will be eligible to enter or make edits to the data. Qualified research personnel will accurately enter data from clinic source documents into the eCRFs provided for this study. Data will be entered into the eCRF shortly after each subject's visit. Study center personnel will exercise due diligence to ensure that study data are entered accurately and in their entirety from the study center's source documents into the appropriate data fields.

The Investigator must review all data entries on a regular basis for completeness and accuracy. When changes or corrections are made to existing entries in the EDC system, the reason for the change must be clearly delineated. The Investigator agrees to transfer study data into the EDC system in a timely fashion and to make the records available to the study monitor for full inspection. In addition, data queries should be answered promptly.

Although the study eCRF is the primary database for the study, all data entered into the eCRF must be recorded in the source documents, and any missing data must be explained. Source data will be retained by the study center as described in Section 10.13.

At the end of the study, by electronically signing the eCRFs the Investigator is attesting to his/her responsibility for the quality of all data recorded, as well as attesting that the data represents a complete and accurate record of each subject's participation in the study.

11.6.2. Source Documentation

Source documents are considered to be all information in original records and certified copies of original records of clinical findings, observations, data or other activities in a clinical study necessary for the reconstruction and evaluation of the study.

The Investigator agrees to allow inspections of the study site and any source documentation, by clinical research and audit personnel from the Sponsor or designee, external auditors or representatives of regulatory authorities. Direct access to the subject's medical/clinical records is necessary to verify and corroborate the data recorded on the eCRFs.

11.7. Study Monitoring

The Sponsor is responsible to regulatory authorities for ensuring the proper conduct of the study regarding protocol adherence and validity of the data recorded on the eCRFs. The Sponsor (or its designee) has therefore assigned study monitors and Medical Monitors to this study. The duties of these monitors are to aid the Investigator and the Sponsor in the maintenance of complete, legible, well-organized, and easily retrievable data. In addition, a monitor will explain and ensure the Investigator's understanding of all applicable regulations concerning the clinical evaluation of a pharmaceutical product (whether licensed or unlicensed) and ensure an understanding of the protocol, reporting responsibilities, and the validity of the data.

In order to perform their role well, the monitors must be given direct access to primary subject data that support data on the eCRFs for the study (e.g., hospital and general practice charts, appointment books, original laboratory records). The Investigator must exercise judgment regarding information in a subject's chart that is not relevant to the performance, observations, or conduct of this study. The Investigator must make available such records to the Sponsor, designated contract research organization (CRO), quality assurance (QA), IRB/IEC, and regulatory personnel for inspection and copying. Because this enters into the realm of subject confidentiality, this fact must be included in the information signed by the subject.

The Investigator should agree, as a minimum requirement, to record the following information in the subject notes:

- Subject identification number
- Protocol identification number
- Date that the subject gave written informed consent
- All visit dates
- All AEs
- All concomitant medications

Entries in the subject notes must contain the signature or initials of the person making the entries, and the date that the entry was made.

The study monitor will perform source-data verification at each monitoring visit.

11.8. Quality Assurance and Auditing Procedures

To ensure compliance with GCP and applicable regulations, the Sponsor may conduct a QA audit of the study center, including inspection of study-center processes and study-related documents.

The Investigator will permit the Sponsor representatives and regulatory authorities to conduct inspections before, during, or after completion of the study. If a regulatory authority requests an inspection, the Investigator must immediately inform the Sponsor of the request.

As appropriate, the Sponsor Clinical QA may be involved in ensuring protocol compliance through auditing activities as well as tracking resolution of any Corrective and Preventive Actions (CAPA) that may be required.

11.8.1. Access to Source Documentation

Source data are all original records of clinical findings, observations, or other activities in a clinical study, which are necessary to achieve study objectives and protect subject safety.

Source data are contained in source documents. Examples of these original documents and data records include:

- Hospital records
- Clinical and office charts
- Laboratory notes
- Memoranda
- Pharmacy dispensing records
- Recorded data from automated instruments
- Copies or transcriptions certified after verification as being accurate and complete
- X-rays, microfiches, photographic negatives, microfilm, or magnetic media
- Subject files, including records kept at the pharmacy, laboratories, and at medico-technical departments involved in the clinical study

Source documents are the original documents used by the Investigator or hospital/institution that allows verification of the existence of the subject and substantiates the integrity of data collected during the study. Source documents will be available to support all data recorded in the eCRF, unless otherwise specified in the eCRF. The Investigator must allow designated representatives of the Sponsor and regulatory inspectors to have direct access to the source documents to verify the data reported in the eCRFs.

The Investigator must maintain source documents for each subject in the study, including source documents that are generated by the subject. All information in the eCRFs must be traceable to these source documents, which are generally maintained in the subject's file. The source documents should contain all demographic and medical information as well as a copy of the signed ICFs provided by the subject.

11.9. Policy on Research Misconduct in Clinical Research

In accordance with GCP, it is the Sponsor's policy always to investigate suspected cases of research misconduct.

11.10. Use of Information and Publication Policy

All information regarding the study drug supplied by the sponsor to the investigator is privileged and confidential information. The investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the sponsor. It is understood that there is an obligation to provide the sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of the study drug and may be disclosed to regulatory authorities, other investigators, corporate partners, or consultants as required. A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law, www.ClinicalTrialsregister.eu, and http://reec.aemps.es. This Web sites will not include information that can identify patients, but may include a summary of the results.

All information concerning the study drug is considered confidential and shall remain the sole property of the sponsor. The investigator(s) agree to use this information only in conducting the study and shall not use it for any other purposes without the sponsor's written approval. The investigator(s) agree not to disclose the sponsor's confidential information to anyone except to persons involved in the study that need such information to assist in conducting the study, and then only on like terms of confidentiality and non-use.

It is understood by the investigator(s) that the information developed from this clinical study will be used by the sponsor in connection with the development of the study drug, and therefore may be disclosed as required to competent authorities. To allow for the use of the information derived from clinical studies, it is understood that there is an obligation to provide the sponsor with complete test results and all data developed in the study. No publication or disclosure of study results will be permitted except as specified in a separate, written, agreement between the sponsor and the investigator(s).

11.11. Amendment to Protocol

Approval of a protocol amendment by the Investigator's IRB/IEC must be obtained before implementation of the protocol amendment, with the following exceptions:

- When necessary to eliminate apparent immediate hazard to the subject
- When the change involves logistical or administrative aspects of the study

The protocol amendment must be signed and dated by both the Sponsor and the Investigator. The Sponsor will submit protocol amendments to the appropriate regulatory authorities (if required)/Ethics Committee (if required) and notify other Investigators using this protocol.

11.12. Deviations from Protocol

Departures from a written protocol for individual subjects are inherent to clinical research and are categorized by the Sponsor as deviations from protocol. A major deviation is a departure

from the protocol which may have impact on the safety or protection of the subject(s), or on the overall quality of the data, and is of sufficient significance to warrant Corrective Action and Preventive Action (CAPA). Minor deviations may appear to be of little or no consequence, but nonetheless they should be reported so that they can be assessed for their effect on the analysis. Examples of deviations include the following:

- Failure to obtain informed consent
- Violation of inclusion/exclusion criteria
- Failure to report SAEs
- Drug dispensing/dosing errors
- Administration of an excluded concomitant medication during the course of the study
- PK-blood sampling time deviations
- PD-blood sampling time deviations

The IRB/IEC will be informed of protocol deviations in a timely manner that is consistent with the IRB's requirements.

The same protocol deviation that occurs for multiple subjects must be recorded separately for each subject.

The Investigator should contact the Sponsor if continuing the subjects in the study is called into question due to a protocol deviation.

11.13. Records of Study

The Investigator will retain essential study documents for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or for at least 2 years after the formal discontinuation of clinical development of the study drug, USL311. Examples of essential documents include the following:

- IRB/IEC correspondence indicating approval/favorable opinion of the study protocol, ICFs, and all amendments to either of these documents
- All source documents and laboratory records
- ICFs signed by the subject
- Subject assent forms, when applicable
- Completed Form FDA 1572
- Statement of Investigator

If required by the applicable regulatory authorities or by an agreement with the Sponsor, these documents should be retained for a longer period (approximately 15 years). The Investigator must notify the Sponsor if he/she changes his/her study-center address or plans to transfer these documents to another Investigator.

11.14. Completion of Study

It is agreed that the Sponsor may terminate this study before the expiration of the agreed upon time period, provided a written notice is submitted at a reasonable time in advance of intended termination.

11.15. Study Funding

The costs necessary to perform the study will be agreed with the Investigator and/or the management of the study facility, and will be documented in a separate financial agreement that will be signed by the Investigator and the Sponsor.

11.16. Financial Disclosure

Clinical Investigators are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR § 54. As defined in 21 CFR § 54.2, a Clinical Investigator is a listed or identified Investigator or sub-Investigator who is directly involved in the treatment or evaluation of research subjects. The term also includes the spouse and each dependent child of the Investigator. In addition, Investigators must promptly update financial disclosure information if any relevant changes occur during the course of the investigation and for 1 year following completion of the study.

12. REFERENCES LIST

1. Karcher S, Steiner HH, Ahmadi R, Zoubaa S, Vasvari G, Bauer H, et al. Different angiogenic phenotypes in primary and secondary glioblastomas. International journal of cancer Journal international du cancer. 2006;118(9):2182-2189.
2. Moore MP, Bagley RS, Harrington ML, Gavin PR. Intracranial tumors. The Veterinary clinics of North America Small animal practice. 1996;26(4):759-777.
3. WHO. World Cancer Report 2008: International Agency for Research on Cancer (IARC); 2008 [ISBN 978 92 832 0423 7]. 511]. Available from: http://www.iarc.fr/en/publications/pdfs-online/wcr/2008/index.php.
4. Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. Neuro-oncology. 2014;16 Suppl 4:iv1-63.
5. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. The New England journal of medicine. 2014;370(8):699-708.
6. Wainwright DA, Nigam P, Thaci B, Dey M, Lesniak MS. Recent developments on immunotherapy for brain cancer. Expert opinion on emerging drugs. 2012;17(2):181-202.
7. Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. The Lancet Oncology. 2014;15(9):e395-403.
8. Hou LC, Veeravagu A, Hsu AR, Tse VC. Recurrent glioblastoma multiforme: a review of natural history and management options. Neurosurgical focus. 2006;20(4):E5.
9. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006;444(7120):756-760.
10. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature. 2012;488(7412):522-526.
11. Dirks PB. Brain tumour stem cells: the undercurrents of human brain cancer and their relationship to neural stem cells. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2008;363(1489):139-152.
12. Wang J, Wakeman TP, Lathia JD, Hjelmeland AB, Wang XF, White RR, et al. Notch promotes radioresistance of glioma stem cells. Stem cells. 2010;28(1):17-28.
13. Walker MD, Alexander E, Jr., Hunt WE, MacCarty CS, Mahaley MS, Jr., Mealey J, Jr., et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. Journal of neurosurgery. 1978;49(3):333-343.
14. Walker MD, Green SB, Byar DP, Alexander E, Jr., Batzdorf U, Brooks WH, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. The New England journal of medicine. 1980;303(23):1323-1329.
15. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. The New England journal of medicine. 2005;352(10):987-996.

16. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. The Lancet Oncology. 2009;10(5):459-466.
17. Ammirati M, Galicich JH, Arbit E, Liao Y. Reoperation in the treatment of recurrent intracranial malignant gliomas. Neurosurgery. 1987;21(5):607-614.
18. Barker FG, 2nd, Chang SM, Gutin PH, Malec MK, McDermott MW, Prados MD, et al. Survival and functional status after resection of recurrent glioblastoma multiforme. Neurosurgery. 1998;42(4):709-20; discussion 20-23.
19. NCCN Guidelines. NCCN Clinical Practice Guidelines in Oncology. Available from: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.
20. Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(26):3212-3218.
21. Wick W, Puduvalli VK, Chamberlain MC, van den Bent MJ, Carpentier AF, Cher LM, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(7):1168-1174.
22. Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. The Journal of experimental medicine. 2005;201(8):1307-1318.
23. Pitchford SC, Furze RC, Jones CP, Wengner AM, Rankin SM. Differential mobilization of subsets of progenitor cells from the bone marrow. Cell stem cell. 2009;4(1):62-72.
24. Gelmini S, Mangoni M, Serio M, Romagnani P, Lazzeri E. The critical role of SDF-1/CXCR4 axis in cancer and cancer stem cells metastasis. Journal of endocrinological investigation. 2008;31(9):809-819.
25. Wang J, Loberg R, Taichman RS. The pivotal role of CXCL12 (SDF-1)/CXCR4 axis in bone metastasis. Cancer metastasis reviews. 2006;25(4):573-587.
26. Ben-Baruch A. Organ selectivity in metastasis: regulation by chemokines and their receptors. Clinical & experimental metastasis. 2008;25(4):345-356.
27. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Seminars in cancer biology. 2004;14(3):171-179.
28. Zhao H, Guo L, Zhao H, Zhao J, Weng H, Zhao B. CXCR4 over-expression and survival in cancer: a system review and meta-analysis. Oncotarget. 2015;6(7):5022-5040.
29. Bian XW, Yang SX, Chen JH, Ping YF, Zhou XD, Wang QL, et al. Preferential expression of chemokine receptor CXCR4 by highly malignant human gliomas and its association with poor patient survival. Neurosurgery. 2007;61(3):570-8; discussion 8-9.
30. Du Y, Long Q, Guan B, Mu L. Prognostic Value of High CXCR4 Expression in Renal Cell Carcinoma: A System Review and Meta-Analysis. Disease markers. 2015;2015:568980.

31. Hu L, He C. Detecting hepatocellular carcinoma in blood. Cell research. 2015;25(12):1279-1280.
32. Amara S, Chaar I, Khiari M, Ounissi D, Weslati M, Boughriba R, et al. Stromal cell derived factor-1 and CXCR4 expression in colorectal cancer promote liver metastasis. Cancer biomarkers : section A of Disease markers. 2015;15(6):869-879.
33. Liu J, Jin J, Yang Y, Han W, Yu W, Shen C, et al. Prognostic Impact of ALDH1 Expression in Transitional Cell Carcinoma of the Renal Pelvis. Anticancer research. 2015;35(9):4829-4836.
34. Zhang C, Li J, Han Y, Jiang J. A meta-analysis for CXCR4 as a prognostic marker and potential drug target in non-small cell lung cancer. Drug design, development and therapy. 2015;9:3267-3278.
35. Xu TP, Shen H, Liu LX, Shu YQ. The impact of chemokine receptor CXCR4 on breast cancer prognosis: a meta-analysis. Cancer epidemiology. 2013;37(5):725-731.
36. Wu J, Wu X, Liang W, Chen C, Zheng L, An H. Clinicopathological and prognostic significance of chemokine receptor CXCR4 overexpression in patients with esophageal cancer: a meta-analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(4):3709-3715.
37. Chen Q, Zhong T. The association of CXCR4 expression with clinicopathological significance and potential drug target in prostate cancer: a meta-analysis and literature review. Drug design, development and therapy. 2015;9:5115-5122.
38. Ehtesham M, Min E, Issar NM, Kasl RA, Khan IS, Thompson RC. The role of the CXCR4 cell surface chemokine receptor in glioma biology. Journal of neuro-oncology. 2013;113(2):153-162.
39. Stevenson CB, Ehtesham M, McMillan KM, Valadez JG, Edgeworth ML, Price RR, et al. CXCR4 expression is elevated in glioblastoma multiforme and correlates with an increase in intensity and extent of peritumoral T2-weighted magnetic resonance imaging signal abnormalities. Neurosurgery. 2008;63(3):560-569; discussion 569-570.
40. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. The Journal of clinical investigation. 2010;120(3):694-705.
41. do Carmo A, Patricio I, Cruz MT, Carvalheiro H, Oliveira CR, Lopes MC. CXCL12/CXCR4 promotes motility and proliferation of glioma cells. Cancer biology & therapy. 2010;9(1):56-65.
42. Redjal N, Chan JA, Segal RA, Kung AL. CXCR4 inhibition synergizes with cytotoxic chemotherapy in gliomas. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006;12(22):6765-6771.
43. Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K, et al. A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(23):13513-13518.
44. Dar A, Schajnovitz A, Lapid K, Kalinkovich A, Itkin T, Ludin A, et al. Rapid mobilization of hematopoietic progenitors by AMD3100 and catecholamines is mediated

by CXCR4-dependent SDF-1 release from bone marrow stromal cells. Leukemia. 2011;25(8):1286-1296.

45. Fan QW, Cheng C, Hackett C, Feldman M, Houseman BT, Nicolaides T, et al. Akt and autophagy cooperate to promote survival of drug-resistant glioma. Science signaling. 2010;3(147):ra81.
46. Ampyra (dalfampridine) Extended Release Tablets [Package Insert]. Ardsley, NY: Acorda Therapeutics, Inc. 2015.
47. Cornblath DR, Bienen EJ, Blight AR. The safety profile of dalfampridine extended release in multiple sclerosis clinical trials. Clinical therapeutics. 2012;34(5):1056-1069.
48. Vivanco I, Robins HI, Rohle D, Campos C, Grommes C, Nghiemphu PL, et al. Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. Cancer discovery. 2012;2(5):458-471.
49. Gilbert MR, Kuhn J, Lamborn KR, Lieberman F, Wen PY, Mehta M, et al. Cilengitide in patients with recurrent glioblastoma: the results of NABTC 03-02, a phase II trial with measures of treatment delivery. Journal of neuro-oncology. 2012;106(1):147-153.
50. Clarke JL, Ennis MM, Yung WK, Chang SM, Wen PY, Cloughesy TF, et al. Is surgery at progression a prognostic marker for improved 6-month progression-free survival or overall survival for patients with recurrent glioblastoma? Neuro-oncology. 2011;13(10):1118-1124.
51. Basch E, Prestrud AA, Hesketh PJ, Kris MG, Feyer PC, Somerfield MR, et al. Antiemetics: American Society of Clinical Oncology clinical practice guideline update. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29(31):4189-4198.
52. Smith TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(19):3187-3205.
53. Reasor MJ, Kacew S. Drug-induced phospholipidosis: are there functional consequences? Experimental biology and medicine. 2001;226(9):825-830.
54. FDA. The Regulatory challenges of drug-induced phospholipidosis. 14 April 2010. p. 112.
55. MOZOBIL (plerixafor solution). Package Insert, Sanofi-aventis U.S. LLC. 2015. p. 16.
56. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European journal of cancer. 2009;45(2):228-247.
57. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. Journal of the National Cancer Institute. 2000;92(3):205-216.
58. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in

neuro-oncology working group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(11):1963-1972.

59. Schag CC, Heinrich RL, Ganz PA. Karnofsky performance status revisited: reliability, validity, and guidelines. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1984;2(3):187-193.
60. Osoba D, Aaronson NK, Muller M, Sneeuw K, Hsu MA, Yung WK, et al. The development and psychometric validation of a brain cancer quality-of-life questionnaire for use in combination with general cancer-specific questionnaires. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 1996;5(1):139-150.
61. Galsky MD, Vogelzang NJ, Conkling P, Raddad E, Polzer J, Roberson S, et al. A phase I trial of LY2510924, a CXCR4 peptide antagonist, in patients with advanced cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(13):3581-3588.
62. Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, et al. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. Antimicrobial agents and chemotherapy. 2000;44(6):1667-1673.
63. Tengstrand EA, Miwa GT, Hsieh FY. Bis(monoacylglycerol)phosphate as a non-invasive biomarker to monitor the onset and time-course of phospholipidosis with drug-induced toxicities. Expert Opin Drug Metab Toxicol. 2010;6(5):555-570.
64. Liu N, Tengstrand EA, Chourb L, Hsieh FY. Di-22:6-bis(monoacylglycerol)phosphate: A clinical biomarker of drug-induced phospholipidosis for drug development and safety assessment. Toxicology and Applied Pharmacology. 2014(279):467-476.
65. Carmichael J, Harris AL. The cardiovascular safety of high-dose intravenous granisetron in cancer patients receiving highly emetogenic chemotherapy. Cancer chemotherapy and pharmacology. 2004;53(2):123-128.
66. Mason JW, Moon TE. Use and cardiovascular safety of transdermal and other granisetron preparations in cancer management. Cancer management and research. 2013;5:179-185.

PROTOCOL SIGNATURE PAGE

Protocol Title: A Phase 1/2 Dose-escalation of USL311 as Single Agent and in Combination with Lomustine (CCNU) in Subjects with Advanced Solid Tumors, with Subsequent Single Agent and Combination Phase 2 Cohorts for Subjects with Relapsed/Recurrent Glioblastoma Multiforme (GBM)

Protocol Number: P311-201

Investigator Agreement: By my signature, I confirm that my staff and I have carefully read and understand this protocol or protocol amendment and agree to comply with the conduct and terms of the study specified herein and with any other study conduct procedures provided by Proximagen, LLC. For protocol amendments, I agree not to implement the amendment without agreement from Proximagen, LLC and prior submission to and written approval (where required) from the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), except when necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements). I agree to conduct the study in accordance with International Conference of Harmonisation E6, Guideline for Good Clinical Practice, and applicable regulatory requirements.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

On behalf of Sponsor, I confirm that Proximagen, LLC, as a sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the Investigator is informed of all relevant information that becomes available during the conduct of this study.

[redacted] 02 AUG 2018

Sponsor's Representative Signature Date

Printed name: [redacted]

APPENDICES

Berry Consultants
Statistical Innovation

Adaptive Design Report for the Phase I Dose Escalation Study of USL311 in Patients with Advanced Solid Tumors Across Two Infusion Times

Submitted to Upsher-Smith

December 22, 2016

1.0 Introduction

This is a Phase I dose-escalation study of USL311 in patients with advanced solid tumors. The primary objective of this trial is to characterize the dose-toxicity relationship and to estimate the maximum tolerated dose (MTD). Dose escalation to determine the MTD will be conducted according to a modified continual reassessment method (mCRM). This design report serves as an update to the initial report to now accommodate two different infusion times. In particular, an additive parameter is added to the dose-toxicity model to estimate the difference in the probability of a grade 2 toxicity or higher as well as the probability of a DLT resulting between the two infusion schedules. A one-time change in the infusion schedule will occur at the sponsor's discretion and all future subjects will be enrolled under the new infusion schedule.

2.0 Single Agent and Combination

USL311 will first be considered as a single agent. This report focuses on the mCRM for dose escalation of USL311 as a single agent. This portion of the trial will enroll a maximum of 40 patients. When dose escalation of USL311 as a single agent is complete, dose escalation for USL311 in combination will be initiated. In combination, only the dose of USL311 will be escalated. This dose escalation will also be conducted according to an mCRM similar to that described here. However, the dose range for the combination will be informed by results of USL311 as a single agent.

3.0 Dose Escalation

3.1 Overview

The primary objective of the dose escalation portion of the study is to estimate an MTD. The MTD will be the highest dose that has a dose-limiting toxicity (DLT) rate less than 33%.

There are a total of 31 possible dose levels between 40 mg/m^2 and 1660 mg/m^2. Dose levels are defined to be in approximately 15% increments. Specifically, for each dose $d = 1,\ldots,31$ the dose levels (v_d), in mg/m^2, are:

40	120	320	820
50	140	360	920
60	160	400	1040
70	180	450	1170
80	200	510	1310
90	220	570	1480
100	250	650	1660
110	280	730	

Dose escalation will be conducted according to a mCRM. Non-DLT toxicity events (i.e. grade 2 toxicities) will inform the probability of DLT at each dose level. Dose escalation is also conducted according to a set of rules that govern entry into the study and assignment of dose level. These rules allow skipping untried dose levels provided they are estimated to be safe. The trial is continuously monitored for safety and for early stopping for successfully identifying the MTD.

3.2 DLT Observation Period

The DLT observation period, for the purpose of dose-escalation, is one cycle. One cycle is 3 weeks in length. Once a patient has completed this first cycle DLT observation period, the patient will be considered to have complete DLT information for the purposes of making dose escalation decisions for the next patient enrolling into the trial. However, DLTs may also appear due to cumulative toxicity, in later cycles. If a DLT appears at any time through the first three cycles of treatment, a patient's DLT status will be updated to reflect the late cycle DLT.

For example, a patient may experience no DLT events in their first cycle. This patient would be recorded as having completed the DLT observation period with no DLT. The next patient enrolled would be assigned a dose level based on that current data. If the patient then experiences a DLT in the second cycle, their DLT status would be updated to reflect the DLT. All future patients enrolled would be assigned a dose level based on data inclusive of that DLT that occurred in the patient's second cycle. In this manner, late cycle DLT information will be taken into account in the dose-toxicity model with the same weight as a first cycle DLT and will inform dose level assignments for the next patients enrolling into the trial.

3.3 Dose-Toxicity Model

For each dose *d = 1,….,31*, we model the ordinal outcome of no toxicity event, non-DLT (i.e. grade 2) toxicity event, or a DLT. We model the log odds of a grade 2 toxicity or higher for dose *d* and infusion schedule *I* (I=0 for initial infusion schedule and 1 for longer infusion schedule),

$$\log\left(\frac{\pi_{\geq grade2}}{1-\pi_{\geq grade2}}\right)=\rho_{d,I}$$

with a three-parameter model

$$\rho_{d,I}=\alpha+\beta(v_d-60)+\theta I\,.$$

Here, we assume that the 60mg/m^2 dose is the referent dose and as such α refers to the log-odds of a grade 2 toxicity or higher under this dose level. The second parameter, β, refers to the log-odds increase in the probability of a grade 2 toxicity or higher per one unit increase/decrease in mg/m^2. The final parameter, θ, refers to the log-odds increase or decrease in the probability of a grade 2 toxicity or higher under the longer infusion schedule.

We model the log odd-odds of a DLT toxicity

$$\log\left(\frac{\pi_{DLT}}{1-\pi_{DLT}}\right)=\mu_{d,I}$$

as related to non-DLTs by including an additive term, γ, which must be less than 0,

$$\mu_{d,I}=(\alpha+\gamma)+\beta(v_d-60)+\theta I.$$

This parameterization assumes that grade 2 toxicities or higher will occur more frequently than the subset of DLT toxicities only and γ defines how much of the total probability of a grade 2 toxicity or higher will be assigned to this subset. We also assume that the additive increase or decrease in the curves attributed to the change in infusion schedule is the same for DLTs and grade 2 toxicities or higher.

We place the following independent prior distributions on the parameters.

$$\alpha \sim \mathrm{N}(-0.5,\, 3);$$
$$\beta \sim \mathrm{N}^{+}(0.004,\, 0.008);$$
$$\gamma \sim \mathrm{N}^{-}(0,\, 2);$$

$$\theta \sim N(0, 1).$$

The model will be updated as frequently as necessary. In particular, the model may be updated as each patient is enrolled into the trial, and/or after each patient is treated and assessed for DLT. When the model is updated, the distributions of all parameters are updated and the MTD per infusion schedule is estimated based on the posterior probability of DLT at each dose.

Additionally, we define a dose as safe if there is at least a 50% probability that the DLT rate is less than 33%,

$$\Pr(\pi_{d,I} < 33\%) > 50\%.$$

This definition for safety is consistent with the maximum likelihood estimate for the probability of DLT. If the DLT rate is estimated to be exactly 33%, the $\Pr(\pi_{d,I} < 33\%)$ will be equal to 50%. Therefore, doses with a mean estimated DLT rate less than 33% will be considered safe by this definition and doses with a mean estimated DLT rate greater than 33% will be considered unsafe by this definition. The upper bound of 33% is justified by the fact that GBM is a highly lethal cancer (has 95% mortality over 5 years) and by the fact that late DLTs will also be counted toward the MTD calculation.

3.4 Dose Escalation Rules

3.4.1 Entry into the Study

Dose escalation begins with enrollment of patients to the 60 mg/m^2 dose. The 40 and 50 mg/m^2 dose levels are de-escalation dose levels. The first three patients will be enrolled as a dosing cohort to the 60 mg/m^2 dose. There must be complete DLT information on these three patients in order to enroll the 4th patient. Starting with the 4th patient, there is open enrollment to the study meaning that patients can be enrolled as they become available for the study. The "queue" refers to the number of patients that have been allocated to doses, but have not yet completed the observation period (one cycle for dose escalation). For safety purposes we want to keep the queue size moderate to prevent large numbers of patients being enrolled at any one time. There may no more than 3 patients enrolled in the trial across the two infusion schedules with uknown DLT information at any time. Patients cannot be enrolled for dose escalation if the queue maximum has been reached.

3.4.2 Assignment of Dose Levels

The dose-toxicity model under the currently enrolling infusion schedule will be used to determine which doses are safe and to assign patients to doses, i.e. to assign patients to the highest safe dose. However, assignment of dose levels is also governed by rules concerning the speed of dose escalation and rules that determine what untried dose levels may be skipped.

When the dose is escalated, it may only escalate to the highest safe dose that is no more than a 100% increase over the current dose level. If the dose must be de-escalated, the dose will de-escalate to the highest safe dose regardless of how large a decrease in dose level. Within an infusion schedule, as long as no DLT has yet been observed there must be complete DLT information on at least 2 patients in order to escalate. Once the first DLT is observed within an infusion schedule, there must be complete DLT information on at least 3 patients within in order to escalate. These rules are applied to each infusion schedule separately. Therefore, if a DLT is observed and then the longer infusion schedule is implemented, the trial may escalate within the longer schedule with 2 patients with complete information per dose level. Once a DLT is observed under the longer schedule, 3 patients complete at each dose level will be required.

3.5 Interim Monitoring

Dose escalation will be continuously monitored for safety and for success in identifying the MTD. In this section, we describe the pre-specified rules to stop the dose-escalation early. If the dose-escalation is not stopped early, dose escalation will stop when the maximum of 40 patients has been enrolled. At that time, the MTD will be the high safe dose as estimated by the dose toxicity model.

3.5.1 Safety Monitoring

If no doses are safe under the current infusion schedule, dose escalation will stop and no MTD will be declared. Formally, if the dose de-escalates to the 40 mg/m^2 dose level, and we have observed at least one patient with complete DLT information at that dose level, and

$$\Pr(\pi_{d,I} < 33\%) < 25\% \text{ for all } d = 1,\ldots 31$$

then the trial will stop early for safety.

3.5.2 Success in Identifying the MTD

Dose escalation may be stopped early when we are sufficiently confident the MTD has been identified within the currently enrolling infusion schedule. We characterize this by having either estimated the MTD with high probability, or by having a sufficient number of patients with complete DLT information at and around the MTD. The trial may stop early for success in identifying the MTD if any one of the following three conditions are satisfied

1. At least 10 patients across infusion schedules on the estimated MTD within the current infusion schedule have complete DLT information

2. At least 15 patients across infusion schedules on the estimated MTD within the current infusion schedule +/- one dose level have complete DLT information

3. There is at least an 80% probability that the current highest safe dose level +/- one dose level is the MTD within the current infusion schedule

4.0 Operating Characteristics

For the purpose of simulation assume the following observed current trial data under the shorter infusion schedule:

- Dose 60: 3 subjects complete with 0 DLTs and 0 grade 2 toxicities
- Dose 120: 4 subjects complete with 0 DLTs and 4 grade 2 toxicities
- Dose 180: 1 subject complete without a DLT and with a grade 2 toxicity

We also assume that a one-time change in the infusion schedule will occur when a DLT is observed and that and all future patients enrolled will be treated at a longer infusion schedule. Once the change occurs, all dose-escalation decisions will be conducted using the dose-toxicity model estimates under the longer infusion schedule.

To evaluate how the dose escalation design performs under the change in infusion schedule, we simulated the study considering different dose-toxicity scenarios under the longer infusion schedule (**Figure 4.1**). The MTD for the shorter infusion schedule is assumed to be 280 for each scenario since under the currently observed data the MTD is estimated to be 280. For each scenario, we show the operating characteristics of the trial based on 1,000 simulations per scenario. Plots show the sample size distribution and the cumulative probability of MTD across the 31 dose levels. The true DLT scenario is shown for reference along with a line indicating a 33% DLT rate.

We summarize the performance of the dose escalation design by showing the mean sample size, the mean number of DLTs, the mean number of patient treated within one dose level of the MTD, and the probability of selecting a dose within one dose level of the true MTD as the MTD.

Table 4.1 provides the following operating characteristics:

- Mean number of total patients enrolled
- Mean number of observed DLTs
- Mean number of patients enrolled to the true MTD +/- 1 dose level
- Probability the true MTD +/- 1 dose level is selected as the most likely MTD or for Scenario 4 where all doses are considered unsafe this is the probability that no MTD is selected.

Table 4.1: Summary Operating Characteristics					
Scenario (MTD Shorter / Longer)	Mean Patients		Mean Number of DLTs	Mean Number of Patients at MTD +/- 1 Dose Level	Probability Selecting true MTD +/- 1 Dose Level
	Total	Longer Infusion Schedule			
1 (280/280)	32	20	8	11	0.60
2 (280 / 400)	34	21	7	11	0.75
3 (280 / 510)	35	22	7	12	0.88
4 (280 / 650)	35	23	7	12	0.88

In Scenario 1 the MTD under both the shorter and longer infusion schedules is 280mg/m^2. On average the trial enrolls 32 total patients (20 under the longer infusion schedule) with an average of 8 DLTs observed among these patients. A mean of 11 patients are enrolled to 280 mg/m^2 +/- one dose level and the likelihood that we choose a dose within this window as the MTD at the end of the trial is 0.60. The probability that we select an unsafe dose above this window as the MTD is 0.10 and the probability that we select a non-optimal dose that is below this window is 0.30.

In Scenario 2-4 the MTD under the longer infusion schedule is 400-650 mg/m2. On average the trials enroll approximately 34-35 patients (21-23 are under the longer infusion schedule). A mean of 11-12 patients are enrolled at the MTD +/- one dose level and the likelihood that we choose a dose within this window as the MTD at the end of the trial is 0.75-0.88. The probability that we select an unsafe dose above this window as the MTD is less than 5% and the probability that we select a non-optimal dose that is below this window is 10-20%. Finally, the probability that the trial stops for futility and no dose is considered safe is less than 0.02.

Figures 4.2-4.5 show more detailed operating characteristics for each scenario. The left panel of each plot below the red curve shows the true probability of DLT. The horizontal dashed line is at 33% for reference. The black curve shows the cumulative probability of DLT. The blue vertical lines show the mean number of patients enrolled at each dose and the blue rectangle shows the mean number of patients treated at the true MTD +/- one dose level. The right panel shows the distribution of the total sample size across the 1000 simulated trials. Dashed vertical line shows the mean total sample size.

Conclusions

This CRM design incorporates the ability to change the infusion schedule to a longer duration and appropriately model the shift in the dose-toxicity relationship that occurs. This is a revision to the dose-toxicity modeling only, and does not impact the conduct of the trial for the patients who have already been enrolled or change the dose levels they would have been assigned to, nor does it impact the conduct of the trial for any future patients to be enrolled under the first, shorter, infusion schedule.

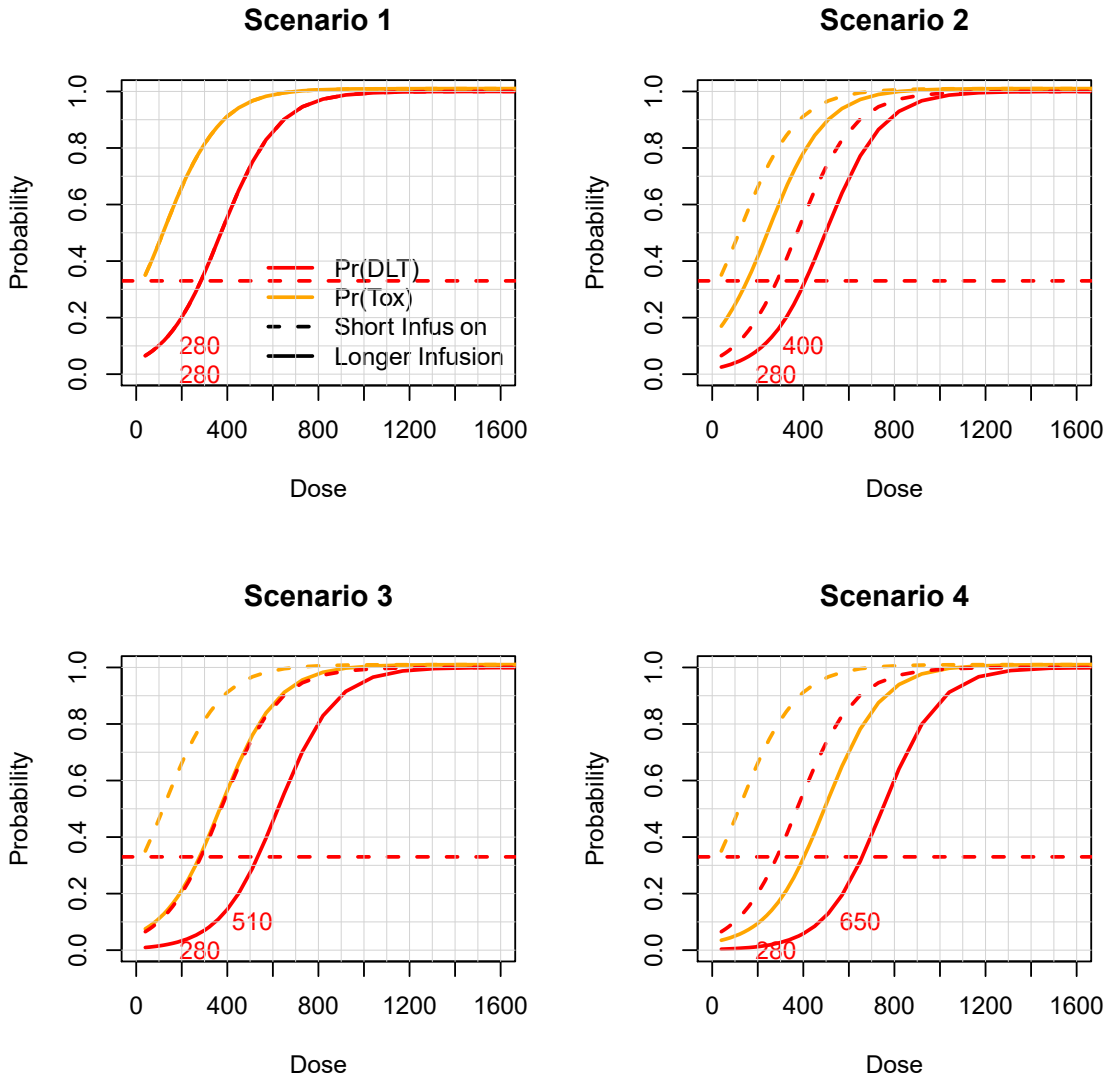


***Figure 4.1**: Dose toxicity scenarios. Red lines correspond to the probability of a DLT, orange lines correspond to the probability of a grade 2 toxicity or higher. Solid lines represent dose-toxicity curves under the longer infusion schedule and dotted lines under the short infusion schedule.*

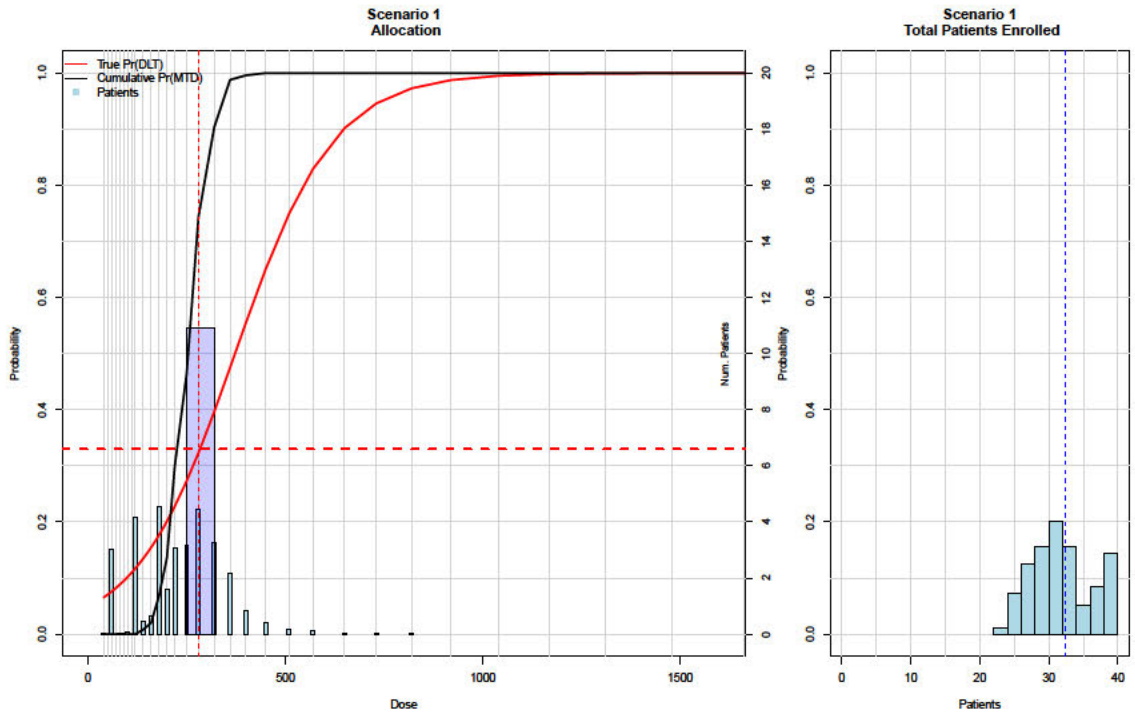


***Figure 4.2**: Operating characteristics for Scenario 1. Left plot: Red solid line represents the true probability of a DLT under the scenario. The black solid line represents the cumulative probability of selecting each dose as the MTD. The blue vertical bars represent the average sample size at each dose and the larger blue shaded bar represents the average sample size at the true MTD +/- 1 dose level. Right plot: Histogram of the total number of patients enrolled. Vertical blue dotted line represents the mean total number of patients enrolled.*

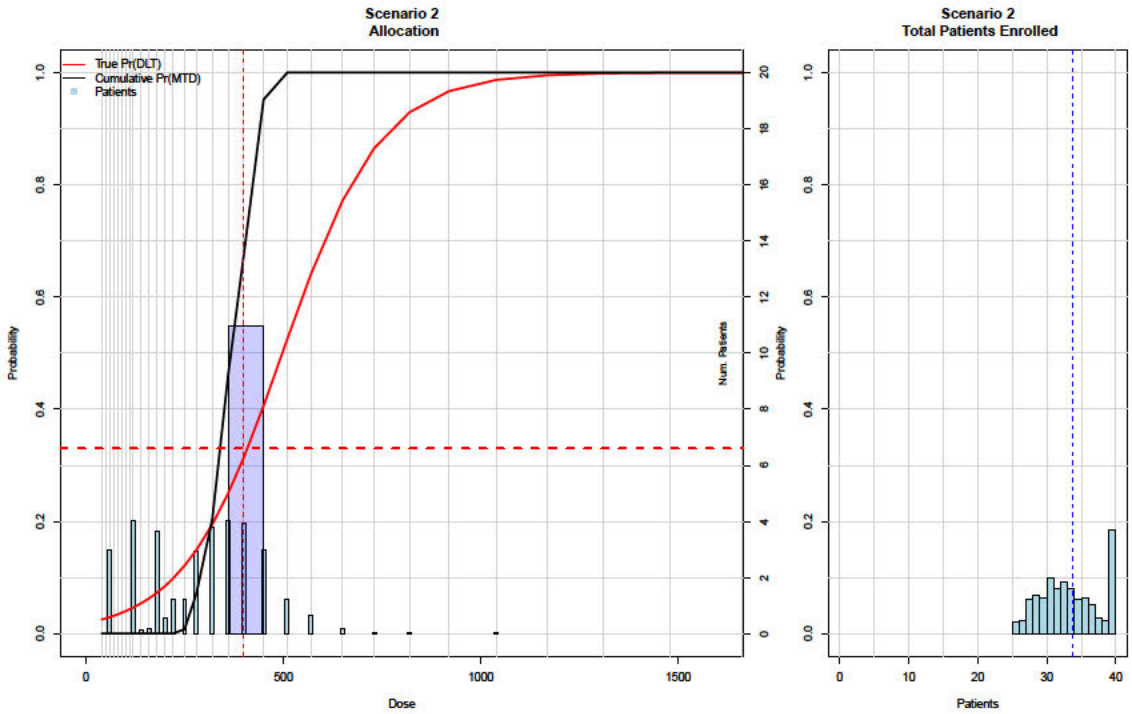


***Figure 4.3**: Operating characteristics for Scenario 2. Left plot: Red solid line represents the true probability of a DLT under the scenario. The black solid line represents the cumulative probability of selecting each dose as the MTD. The blue vertical bars represent the average sample size at each dose and the larger blue shaded bar represents the average sample size at the true MTD +/- 1 dose level. Right plot: Histogram of the total number of patients enrolled. Vertical blue dotted line represents the mean total number of patients enrolled.*

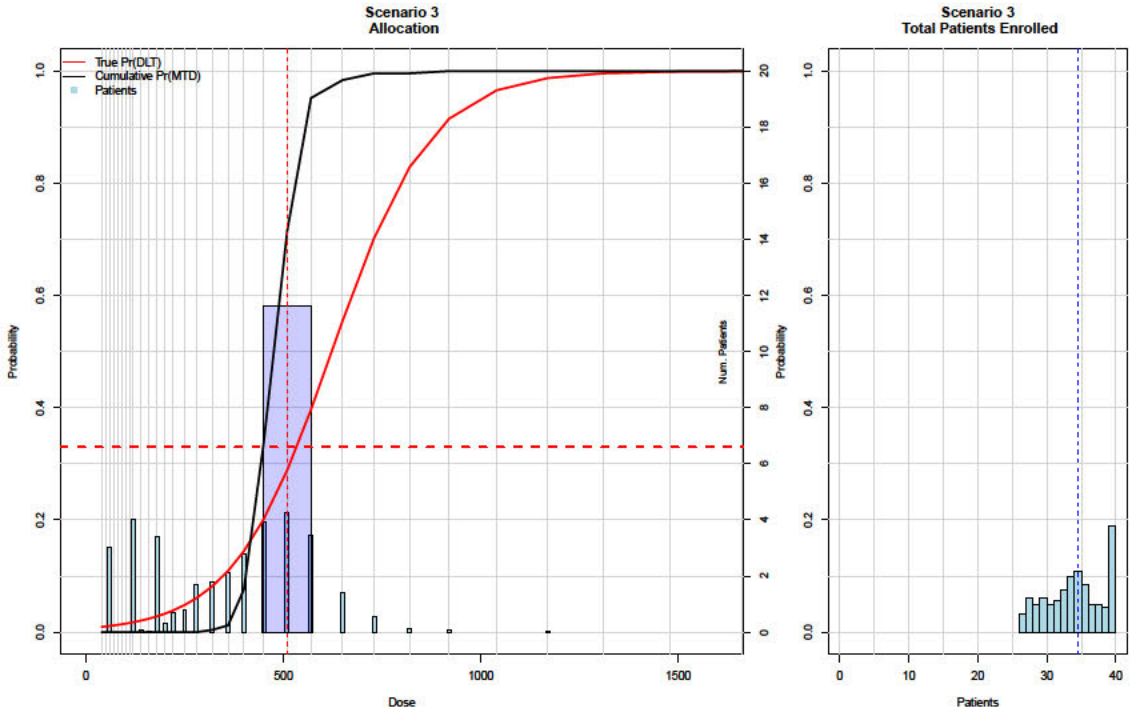


***Figure 4.4**: Operating characteristics for Scenario 3. Left plot: Red solid line represents the true probability of a DLT under the scenario. The black solid line represents the cumulative probability of selecting each dose as the MTD. The blue vertical bars represent the average sample size at each dose and the larger blue shaded bar represents the average sample size at the true MTD +/- 1 dose level. Right plot: Histogram of the total number of patients enrolled. Vertical blue dotted line represents the mean total number of patients enrolled.*

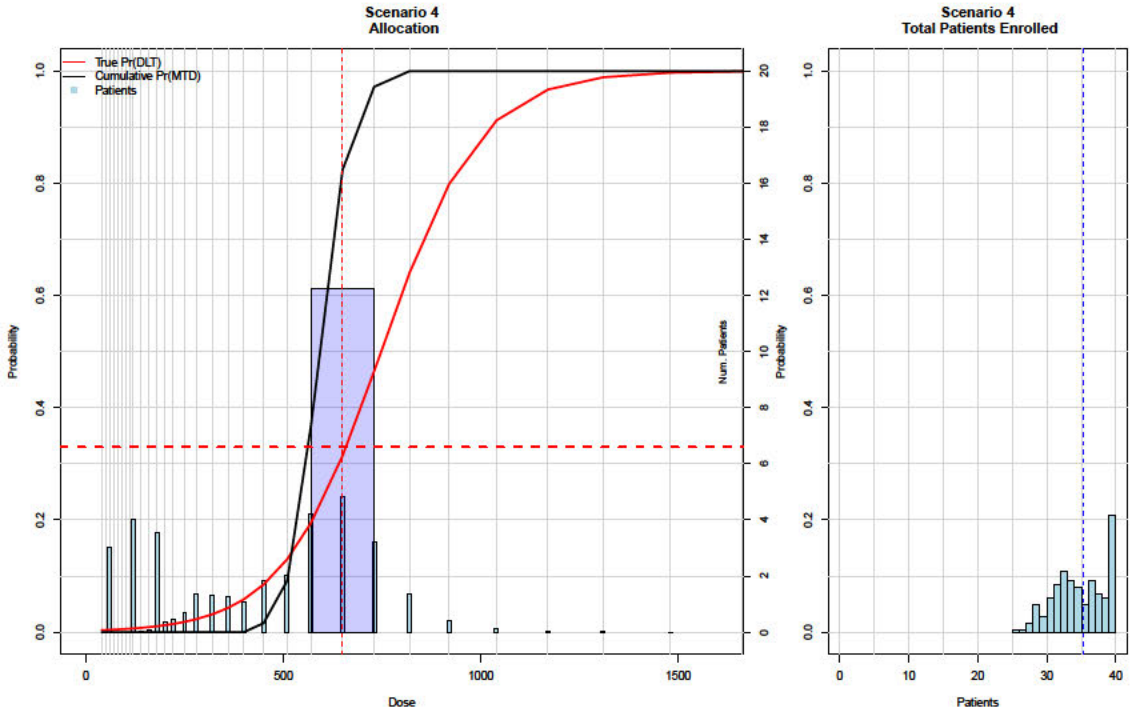


***Figure 4.5**: Operating characteristics for Scenario 4. Left plot: Red solid line represents the true probability of a DLT under the scenario. The black solid line represents the cumulative probability of selecting each dose as the MTD. The blue vertical bars represent the average sample size at each dose and the larger blue shaded bar represents the average sample size at the true MTD +/- 1 dose level. Right plot: Histogram of the total number of patients enrolled. Vertical blue dotted line represents the mean total number of patients enrolled.*

Berry Consultants
Statistical Innovation

Adaptive Design Report for the Phase I Dose Escalation Study of Oral Dosing of USL311 in Patients with Advanced Solid Tumors

Submitted to Upsher-Smith

January 5, 2018

1.0 Introduction

This is a Phase I dose-escalation study of oral dosing of USL311 in patients with advanced solid tumors. The primary objective of this trial is to characterize the dose-toxicity relationship and to estimate the maximum tolerated dose (MTD). Dose escalation to determine the MTD will be conducted according to a modified continual reassessment method (mCRM). This study will enroll a maximum of 40 patients.

2.0 Dose Escalation

2.1 Overview

The primary objective of the dose escalation portion of the study is to estimate an MTD. The MTD will be the highest dose that has a dose-limiting toxicity (DLT) rate less than 33%.

There are a total of 27 possible dose levels between 20 mg and 1440 mg. Dose levels are defined to be at least 15% apart rounded to the nearest 20 mg. Specifically, for each dose $d = 1,\ldots,27$ the dose levels (v_d), in mg, are:

20	180	400	1100
40	200	460	1260
60	220	520	1440
80	240	580	
100	260	660	
120	280	740	
140	320	840	
160	360	960	

Dose escalation will be conducted according to a mCRM. Non-DLT toxicity events (i.e. grade 2 toxicities) will inform the probability of DLT at each dose level. Dose escalation is also conducted according to a set of rules that govern entry into the

study and assignment of dose level. These rules allow skipping untried dose levels provided they are estimated to be safe. The trial is continuously monitored for safety and for early stopping for successfully identifying the MTD.

2.2 DLT Observation Period

The DLT observation period, for the purpose of dose-escalation, is one cycle. One cycle is 3 weeks in length. Once a patient has completed this first cycle DLT observation period, the patient will be considered to have complete DLT information for the purposes of making dose escalation decisions for the next patient enrolling into the trial. However, DLTs may also appear due to cumulative toxicity, in later cycles. If a DLT appears at any time through the first three cycles of treatment, a patients DLT status will be updated to reflect the late cycle DLT.

For example, a patient may experience no DLT events in their first cycle. This patient would be recorded as having completed the DLT observation period with no DLT. The next patient enrolled would be assigned a dose level based on that current data. If the patient then experiences a DLT in the second cycle, their DLT status would be updated to reflect the DLT. All future patients enrolled would be assigned a dose level based on data inclusive of that DLT that occurred in the patient's second cycle. In this manner, late cycle DLT information will be taken into account in the dose-toxicity model with the same weight as a first cycle DLT and will inform dose level assignments for the next patients enrolling into the trial.

2.3 Dose-Toxicity Model

For each dose $d = 1,\ldots,27$, we model the ordinal outcome of no toxicity event, non-DLT (i.e. grade 2) toxicity event, or a DLT. We model the log odds of a grade 2 toxicity or higher with a two-parameter model,

$$log\left(\frac{\pi_{>Grade2,d}}{1-\pi_{>Grade2,d}}\right) = \alpha + \beta(v_d - 40).$$

Here, we assume that the 40mg dose is the referent dose and as such α refers to the log-odds of a grade 2 toxicity or higher under this dose level. The second parameter, β, refers to the log-odds increase in the probability of a grade 2 toxicity or higher per one unit increase/decrease in mg.

We model the log odd-odds of a DLT toxicity as related to non-DLTs by including an additive term, γ, which must be less than 0,

$$log\left(\frac{\pi_{DLT,d}}{1-\pi_{DLT,d}}\right) = \alpha + \gamma + \beta(v_d - 40).$$

This parameterization assumes that grade 2 toxicities or higher will occur more frequently than the subset of DLT toxicities only and γ defines how much of the total probability of a grade 2 toxicity or higher will be assigned to this subset.

We place the following independent prior distributions on the parameters.

$$\alpha \sim \mathrm{N}(\ 2, 1);$$
$$\beta \sim \mathrm{N}_{+}(0.004, 0.008);$$
$$\gamma \sim \mathrm{N}\ (0, 1).$$

Figure 2.1 shows the prior dose-toxicity curve. The solid red line corresponds to the mean probability of a DLT toxicity at each dose. The dotted orange line corresponds to the mean probability of a Grade 2 toxicity event or higher at each dose for each disease cohort. The light shaded red region corresponds to 95% CI for the probability of a DLT toxicity at each dose. The MTD based on the prior distribution is 280 mg.

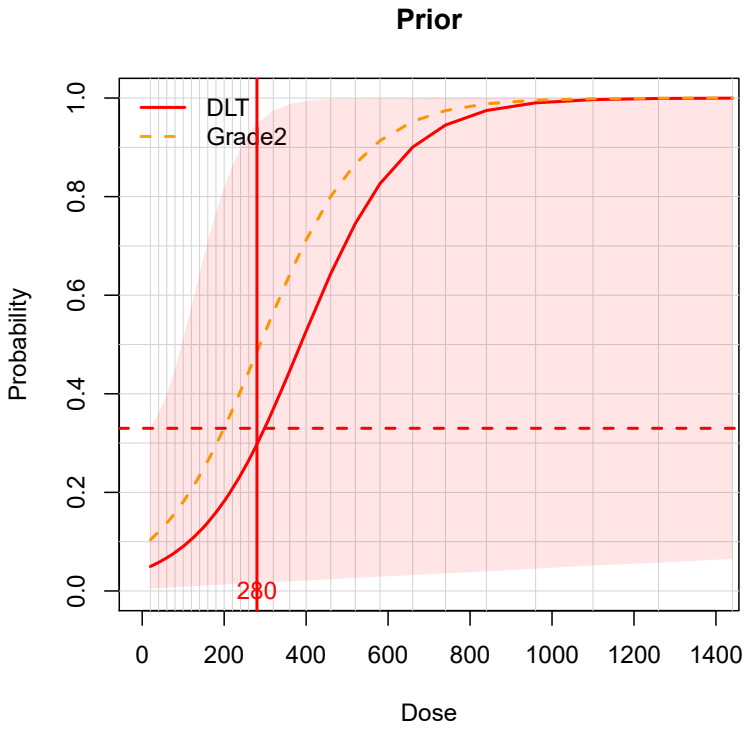


Figure 2.1: Prior dose-toxicity curve. Solid red line represents the mean probability of a DLT at each dose. The light shaded region is the corresponding 95% CI. Dotted orange line represents the mean probability of a Grade 2 toxicity or higher at each dose. The horizontal red dotted line is at a 33% DLT rate for reference.

The model will be updated as frequently as necessary. In particular, the model may be updated as each patient is enrolled into the trial, and/or after each patient is treated and assessed for DLT. When the model is updated, the distributions of all parameters are updated and the MTD is estimated based on the posterior probability of DLT at each dose.

Additionally, we define a dose as safe if there is at least a 50% probability that the DLT rate is less than 33%,

$$\Pr(\pi_d < 33\%) > 50\%.$$

This definition for safety is consistent with the maximum likelihood estimate for the probability of DLT. If the DLT rate is estimated to be exactly 33%, the $\Pr(\pi_d < 33\%)$ will be equal to 50%. Therefore, doses with a mean estimated DLT rate less than 33% will be considered safe by this definition and doses with a mean estimated DLT rate greater than 33% will be considered unsafe by this definition. The upper bound of 33% is justified by the fact that GBM is a highly lethal cancer (has 95% mortality over 5 years) and by the fact that late cycle DLTs will also be counted toward the MTD calculation.

2.4 Dose Escalation Rules

2.4.1 Entry into the Study

Dose escalation begins with enrollment of patients to the 40mg dose. The 20mg dose level is a de-escalation dose level. The first patient will be enrolled to the 40mg dose. Beginning with this first patient enrolled there is open enrollment to the study meaning that patients can be enrolled as they become available for the study. The "queue" refers to the number of patients that have been allocated to doses, but have not yet completed the observation period (one cycle for dose escalation). For safety purposes we want to keep the queue size moderate to prevent large numbers of patients being enrolled at any one time. There may no more than 3 patients enrolled in the trial with unknown DLT information at any time. Patients cannot be enrolled for dose escalation if the queue maximum has been reached.

2.4.2 Assignment of Dose Levels

The dose-toxicity model will be used to determine which doses are safe and to assign patients to doses, i.e. to assign patients to the highest safe dose. However, assignment of dose levels is also governed by rules concerning the speed of dose escalation and rules that determine what untried dose levels may be skipped.

When the dose is escalated, it may only escalate to the highest safe dose that is no more than a 100% increase over the current dose level. If the dose must be de-escalated, the dose will de-escalate to the highest safe dose regardless of how large a decrease in dose level. As long as no DLT has yet been observed, there must be complete DLT information on at least 2 patients at the current dose level in order to

escalate. Once the first DLT is observed in the trial, there must be complete DLT information on at least 3 patients at the current dose level in order to escalate.

2.5 Interim Monitoring

Dose escalation will be continuously monitored for safety and for success in identifying the MTD. In this section, we describe the pre-specified rules to stop the dose-escalation early. If the dose-escalation is not stopped early, dose escalation will stop when the maximum of 40 patients has been enrolled. At that time, the MTD will be the high safe dose as estimated by the dose toxicity model.

2.5.1 Safety Monitoring

If no doses are safe, dose escalation will stop and no MTD will be declared. Formally, if the dose de-escalates to the 20mg dose level, and we have observed at least one patient with complete DLT information at that dose level, and

$$\Pr(\pi_d < 33\%) < 25\% \text{ for all } d = 1,\ldots 31$$

then the trial will stop early for safety.

2.5.2 Success in Identifying the MTD

Dose escalation may be stopped early when we are sufficiently confident the MTD has been identified. We characterize this by having either estimated the MTD with high probability, or by having a sufficient number of patients with complete DLT information at and around the MTD. The trial may stop early for success in identifying the MTD if any one of the following three conditions is satisfied

1. At least 10 patients on the estimated MTD have complete DLT information

2. At least 15 patients on the estimated MTD +/- one dose level have complete DLT information

3. There is at least an 80% probability that the current highest safe dose level +/- one dose level is the MTD and there are at least 3 patients on the estimated MTD with complete DLT information

3.0 Example Trials

In this section we present selected snapshots from single simulated trials to illustrate the dose escalation. Tables show the observed data, including the total number of patients enrolled at each dose level, the number with complete DLT information, the number of grade 2 toxicity events, and the number of DLT events observed. We also show quantities from the dose-toxicity model for each dose level including the probability of DLT, the probability the dose is safe, and the probability

the dose is the MTD. The upper part of each table presents information for each dose level where patients have been enrolled. The lower part of each table highlights the current MTD +/- 1 dose level.

4.1 Example Trial 1

The first four patients are enrolled to 40 mg. At least 2 patients must complete the DLT observation period before the trial can escalate. The fifth and sixth patients are available to be enrolled when all four of the patients enrolled to 40 mg are complete and there are no observed DLTs or Grade 2 toxicities. The dose is escalated 100% to 80 mg and the fifth and sixth patients are enrolled to this dose. The current MTD is estimated to be 360 with a mean DLT rate of 0.31 (95% CI (0.51, 0.56)). (Table 4.1.1).

Table 4.1.1: 5 Patients Enrolled and 4 with Complete DLT Information

		Observed Data				**Dose-Toxicity Model Quantities**		
	Dose Level (mg)	**N Enrolled**	**N Complete**	**N DLT**	**N Grade 2**	**Pr DLT**	**Pr Safe**	**Pr MTD**
Current and Last Dose Levels	40	4	4	0	0	0.03	1.00	0.00
	80	2	0	0	0	0.04	1.00	0.01
Highlight on Current MTD +/- 1 Dose Level	320	0	0	0	0	0.24	0.62	0.06
	360	0	0	0	0	0.31	0.56	0.05
	400	0	0	0	0	0.38	0.49	0.07
	Total For MTD Window	*0*	*0*	*0*	*0*			*0.18*

The next three patients are enrolled to the 160 mg dose after the two patients complete on 80 mg without any DLTs or Grade 2 toxicities. The estimated MTD increases to 400 mg.

All three patients on the 160 mg dose level do not observe a DLT or Grade 2 toxicity, the estimated MTD increase to 520 mg and patients 10-13 are enrolled to dose level 320.

When all four of the patients at the 320 mg dose complete the DLT observation period, one has experienced a grade 2 toxicity and another one has experienced a DLT. The estimated MTD decreases from 520 mg to 360 mg. With a DLT observed, now three patients complete through the DLT observation period are required for dose escalation. There are 4 patients complete at the current dose level and the dose may increase, but no more than 100% or to the highest safe dose. The dose is escalated to the 360 mg dose level and patients 14-16 are enrolled to dose level 360 (Table 4.1.2).

Table 4.1.2: 16 Patients Enrolled and 13 with Complete DLT Information								
		Observed Data				**Dose-Toxicity Model Quantities**		
	Dose Level (mg)	**N Enrolled**	**N Complete**	**N DLT**	**N Grade 2**	**Pr DLT**	**Pr Safe**	**Pr MTD**
Current and Last Dose Levels	320	4	4	1	1	0.20	0.79	0.16
	360	3	0	0	0	0.26	0.63	0.14
Highlight on Current MTD +/- 1 Dose Level	320	4	4	1	1	0.20	0.79	0.16
	360	3	0	0	0	0.26	0.63	0.14
	400	0	0	0	0	0.33	0.49	0.16
	Total for MTD Window	*7*	*4*	*1*	*1*	--	--	*0.45*

The first patient on dose level 360 experiences a DLT and the trial de-escalates to dose level 320. Patient 17 is enrolled to 320 mg. The next patient on dose level 360 also observes a DLT and the trial further de-escalates to dose level 280. Patient 18-20 are all enrolled to 280 mg.

The trial continues enroll patients 21-27 to dose levels 280-320mg. At this point in the trial the MTD is estimated to be 320 mg (DLT rate 0.30) and there are greater than 15 subjects (a total of 18) complete in the MTD window so the trial stops for successfully identifying the MTD. In the MTD window 5/18 subjects experience a DLT and 6/18 experience a Grade 2 toxicity or higher. (Table 4.1.3).

Table 4.1.3: Trial Stops enrollment for success in identifying the MTD								
		Observed Data				**Dose-Toxicity Model Quantities**		
	Dose Level (mg)	**N Enrolled**	**N Complete**	**N DLT**	**N Grade 2**	**Pr DLT**	**Pr Safe**	**Pr MTD**
Current and Last Dose Levels	280	4	4	1	1	0.22	0.91	0.29
	320	11	11	2	5	0.30	0.62	0.35
Highlight on Current MTD +/- 1 Dose Level	280	4	4	1	1	0.22	0.91	0.29
	320	11	11	2	5	0.30	0.62	0.35
	360	3	3	2	0	0.40	0.27	0.17
	Total for MTD Window	*18*	*18*	*5*	*6*	--	--	*0.81*

4.2 Example Trial 2

The first three patients are enrolled to 40 mg by the time the first patient observes a DLT. With one patient complete and one DLT on the 40 mg dose the current estimated DLT rate at 40 mg is 0.23 and this dose is still considered safe. Patient 4 is then enrolled to the 40 mg dose. (Table 4.2.1)

Table 4.2.1: 4 Patients Enrolled; 1 Complete on 40 mg

		Observed Data				Dose-Toxicity Model Quantities		
	Dose Level (mg)	**N Enrolled**	**N Complete**	**N DLT**	**N Grade 2**	**Pr DLT**	**Pr Safe**	**Pr MTD**
Enrolled Dose Levels	40	4	1	1	0	0.23	0.70	0.07

When the second patient enrolled to the 40 mg dose also observes a DLT the trial de-escalates to the 20 mg dose and Patient 5 is enrolled to this dose. The probability that the 20 mg dose is safe is 0.41. If this probability is less than 0.25 we will stop the trial early for futility. (Table 4.2.2)

Table 4.2.2: 4 Patients Enrolled; 2 Complete on 40 mg

		Observed Data				Dose-Toxicity Model Quantities		
	Dose Level (mg)	**N Enrolled**	**N Complete**	**N DLT**	**N Grade 2**	**Pr DLT**	**Pr Safe**	**Pr MTD**
Enrolled Dose Levels	20	1	0	0	0	0.37	0.41	0.08
	40	4	2	2	0	0.41	0.33	0.07

When the patient enrolled to the 20 mg dose also observes a DLT the probability that this dose is safe drops to 0.08 and the trial stops early for futility. The final analysis is shown in Table 4.2.3. All 4 patients on dose level 40 experienced a DLT and the one patient on the 20mg dose experienced a grade 2 toxicity. No dose levels are considered safe.

Table 4.2.3: Final Analysis; 5 Patients Complete on 20 and 40 mg

	Observed Data				Dose-Toxicity Model Quantities		
Dose Level	**N Enrolled**	**N Complete**	**N DLT**	**N Grade**	**Pr DLT**	**Pr Safe**	**Pr MTD**

	(mg)				2			
Enrolled	20	1	1	0	1	0.54	0.08	0.03
Dose Levels	40	4	4	4	0	0.58	0.04	0.02

4.0 Operating Characteristics

To evaluate how the dose escalation design performs, we simulated the study considering different dose-toxicity scenarios (Figure 4.1).

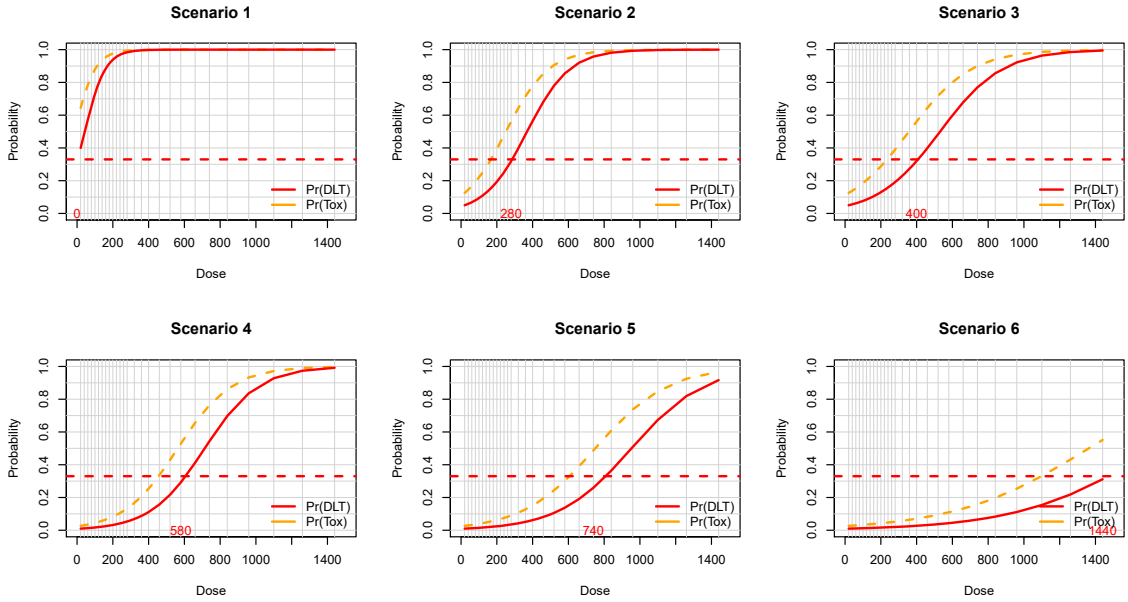


Figure 4.1*: Dose toxicity scenarios*

For each scenario, we show the operating characteristics of the trial based on 1,000 simulations per scenario. Plots show the sample size distribution and the cumulative probability of MTD across the 27 dose levels. The true DLT scenario is shown for reference along with a line indicating a 33% DLT rate. We further summarize the performance of the dose escalation design by showing the mean sample size, the mean number of DLTs, the mean number of patient treated within one dose level of the MTD, and the probability of selecting a dose within one dose level of the true MTD as the MTD in Table 4.1. Figure 4.2 shows the cumulative probability of selecting each dose on each scenario as well as the distribution of simulated trial sizes.

In Scenario 1 all doses are unsafe. On average the trial enrolls approximately 13 patients with an average of 6 DLTs observed among these patients. The likelihood that we claim that all doses are unsafe is 86% with the remaining 14% of trials choosing dose level 20 or 40mg.

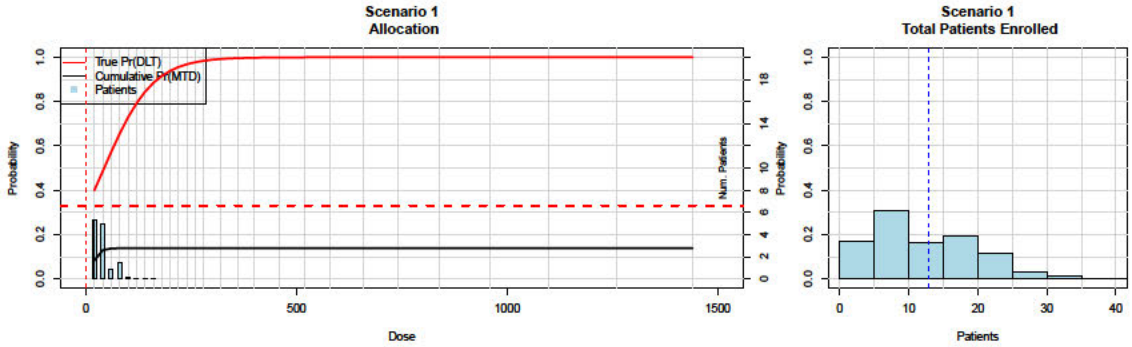
When the MTD is in the middle of the dosing grid (Scenarios 2 and 3 with MTDs of 280 and 400) the trial enrolls on average 33 patients with 7-8 of them experiencing DLTs. The mean number of patients on the true MTD +/- one dose level is 9-10 and the probability of selecting the true MTD +/- one dose level is 0.54 0.57. In both scenarios, there is a 40% chance that we select an MTD less than one dose level below the true MTD and an 3-6% chance of selecting an MTD greater than one dose level above the true MTD.

When the MTD is in the upper end of the dosing grid (Scenarios 3 and 4 with MTDs of 580 and 740) the trial enrolls on average 32 patients with 6 of them experiencing a DLT. The mean number of patients on the true MTD +/- one dose level is 11-12 and the probability of selecting the true MTD +/- one dose level is 0.79-0.85. In Scenarios 4 and 5, the likelihood of selecting an MTD less than one dose level below the true MTD is 18% and 12% respectively and the likelihood of selecting an MTD greater than one dose level above the true MTD 3%.

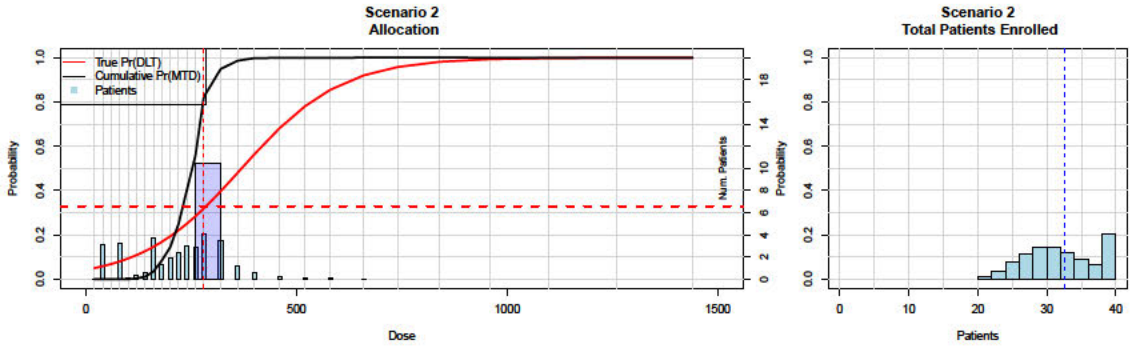
Finally, when the MTD is the maximum dose (1440 mg) the trial enrolls on average 28 patients with approximately 3 of them experiencing a DLT. The mean number of patients on the true MTD or one dose level below (1260) is 6 and the probability of selecting either 1440 or 1260 is 0.87.

Table 4.1: Summary Operating Characteristics

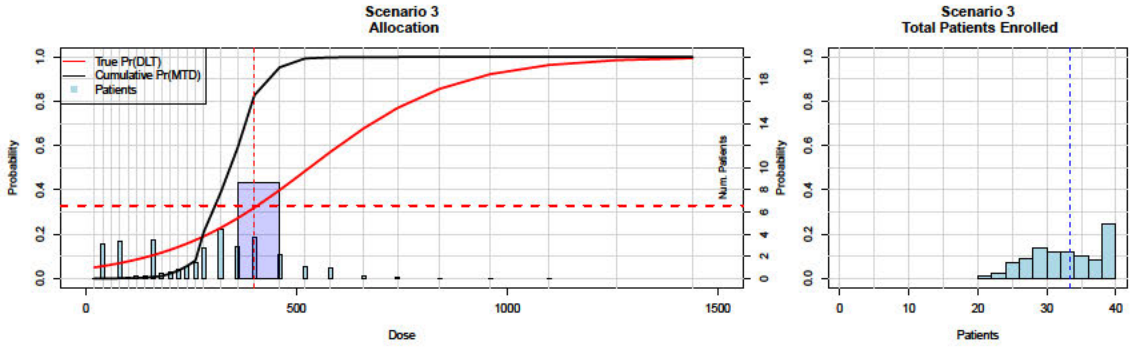
Scenario	True MTD	Mean Total Patients	Mean Number of DLTs	Mean Number of Patients at MTD +/- 1 Dose Level	Probability Selecting true MTD +/- 1 Dose Level
1	0	13	6	-	0.86
2	280	33	8	10	0.54
3	400	33	7	9	0.57
4	580	32	6	12	0.79
5	740	32	6	11	0.85
6	1440	28	3	6	0.87


Scenario 1
Allocation
True Pr(DLT)
Cumulative Pr(MTD)
Patients
Probability
Num. Patients
Dose

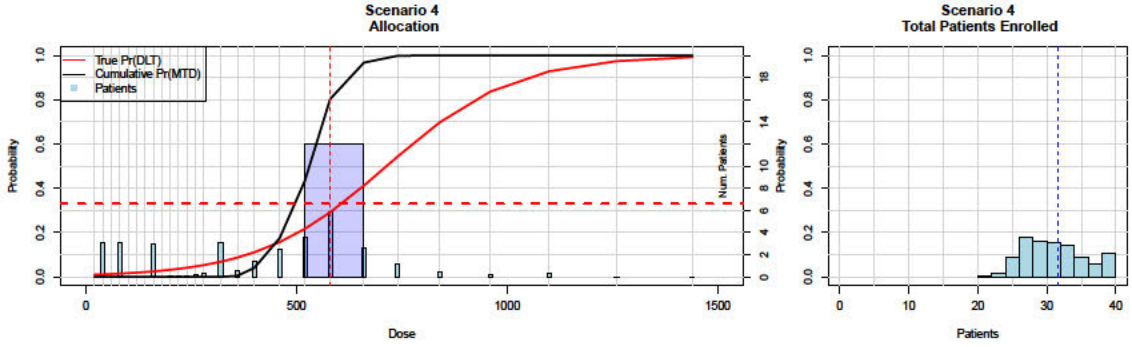
Scenario 1
Total Patients Enrolled
Probability
Patients


Scenario 2
Allocation
True Pr(DLT)
Cumulative Pr(MTD)
Patients
Probability
Num. Patients
Dose

Scenario 2
Total Patients Enrolled
Probability
Patients


Scenario 3
Allocation
True Pr(DLT)
Cumulative Pr(MTD)
Patients
Probability
Num. Patients
Dose

Scenario 3
Total Patients Enrolled
Probability
Patients


Scenario 4
Allocation
True Pr(DLT)
Cumulative Pr(MTD)
Patients
Probability
Num. Patients
Dose

Scenario 4
Total Patients Enrolled
Probability
Patients

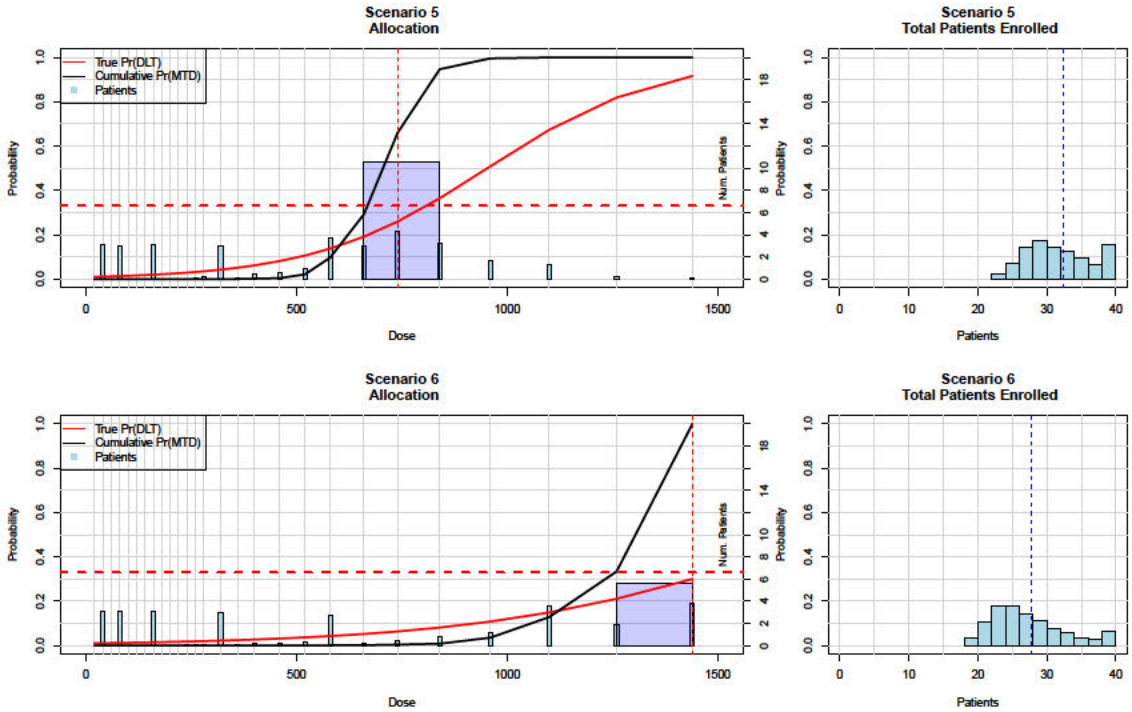


***Figure 4.2**: Operating characteristics for Scenario 1-6. Left panel: The red curve shows the true probability of DLT. The horizontal dashed line is at 33% for reference. The black curve shows the cumulative probability of DLT. The blue vertical lines show the mean number of patients enrolled at each dose and the blue rectangle shows the mean number of patients treated at the true MTD +/- one dose level. Right panel: The distribution of the total sample size across the 1000 simulated trials. Dashed vertical line shows the mean total sample size.*

Appendix 2. Highly Effective Contraceptive Methods

Contraception methods that can achieve a failure rate of < 1% per year when used consistently are considered to be highly effective. Such methods include the following:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation. Note: Hormonal contraception may be susceptible to interaction with the study drug, which may reduce the efficacy of the contraception method.
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation. Note: Hormonal contraception may be susceptible to interaction with the study drug, which may reduce the efficacy of the contraception method.
  - Oral
  - Injectable
  - Implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomized partner
  - Note: Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.
- Sexual Abstinence
  - Note: Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Appendix 3. Medications with QTc Prolongation Risk

High risk QTc prolongation medications are those that demonstrate a prolonged QTc interval after administration and are clearly associated with Torsade de Pointes (TdP), even when used as directed. Moderate risk QTc prolongation medications are those that have substantial evidence of causing QTc prolongation but do not have enough evidence of causing TdP when used as directed. The following table lists the medications with high and moderate QTc prolongation risk:

This list may not be all-inclusive; direct any questions to the Medical Monitor. Additionally, please visit crediblemeds.org for more information.

Appendix 3 – Table 1: Medications with QTc Prolongation Risk

Medication Class	High Risk Medications	Moderate Risk Medications
Antibiotics/ Antifungals	Azithromycin Ciprofloxacin Clarithromycin Erythromycin Fluconazole Gatifloxacin[a] Levofloxacin Moxifloxacin Pentamidine Sparfloxacin[a]	Bedaquiline Delamanid[a] Gemifloxacin Norfloxacin Ofloxacin Roxithromycin[a] Telavancin Telithromycin
Antidepressants	Citalopram Escitalopram	Clomipramine Desipramine Imipramine Mirtazapine Nortriptyline Trimipramine Venlafaxine
Antiemetics/ Antinausea	Domperidone[a] Ondansetron	Dolasetron Granisetron[b] Tropisetron[a]
Antihistamines	Astemizole[a] Terfenadine[a]	
Antimalarials/ Antivirals	Chloroquine Halofantrine	Atazanavir Foscarnet Rilpivirine Saquinavir

Appendix 3 – Table 1: Medications with QTc Prolongation Risk (Continued)

Medication Class	High Risk Medications	Moderate Risk Medications	
Antipsychotics	Chlorpromazine Droperidol Haloperidol Levomepromazine[a] Mesoridazine[a] Pimozide Sulpiride[a] Thioridazine	Aripiprazole Asenapine Clozapine Iloperidone Olanzapine Paliperidone Pipamperone[a] Promethazine Risperidone Sertindole[a]	
Anesthetics	Cocaine (local anesthetic) Propofol Sevoflurane	Dexmedetomidine	
Cardiovascular	Amiodarone Bepridil[a] Disopyramide Dofetilide Dronedarone Flecainide Ibutilide Procainamide (oral)[a] Quinidine Sotalol	Israpidine Moexipril/hydrochlorothiazide Nicardipine Ranolazine	
Phosphodiesterase 3 Inhibitors	Anagrelide Cilostazol		
Opioids	Levomethadyl[a] Methadone	Hydrocodone ER[c]	
Oncologic	Arsenic trioxide Oxaliplatin Vandetanib	Bortezomib Bosutinib Ceritinib Crizotinib Dabrafenib Dasatinib Degarelix Eribulin mesylate Lapatinib Leuprolide	Nilotinib Panobinostat Pazopanib Sorafenib Sunitinib Tamoxifen Toremifene Vemurafenib Vorinostat

Appendix 3 – Table 1: Medications with QTc Prolongation Risk (Continued)

Medication Class	High Risk Medications	Moderate Risk Medications	
Other drugs	Cisapride[a] Donepezil Probucol[a] Papaverine HCL	Alfuzosin Apomorphine Artenimol + piperaquine Atomoxetine Famotidine Felbamate Fingolimod Lithium Mifepristone Mirebegron	Oxytocin Pasireotide Perflutren lipid microspheres Tacrolimus Tetrabenazine Tizanidine Tolterodine Vardenafil

[a] Medication not available in the US
[b] Published literature report minimal or no change to QTc interval with the administration of granisetron (65, 66).
[c] Observed with extended release only

Appendix 4. Lomustine (Gleostine®) Package Insert

Gleostine™
(lomustine) Capsules

WARNINGS

Gleostine (lomustine) should be administered under the supervision of a qualified physician experienced in the use of cancer chemotherapeutic agents.

Bone marrow suppression, notably thrombocytopenia and leukopenia, which may contribute to bleeding and overwhelming infections in an already compromised patient, is the most common and severe of the toxic effects of Gleostine (see **WARNINGS** and **ADVERSE REACTIONS**).

Since the major toxicity is delayed bone marrow suppression, blood counts should be monitored weekly for at least 6 weeks after a dose (see **ADVERSE REACTIONS**). At the recommended dosage, courses of Gleostine should not be given more frequently than every 6 weeks.

The bone marrow toxicity of Gleostine is cumulative and therefore dosage adjustment must be considered on the basis of nadir blood counts from prior dose (see dosage adjustment table under **DOSAGE AND ADMINISTRATION**).

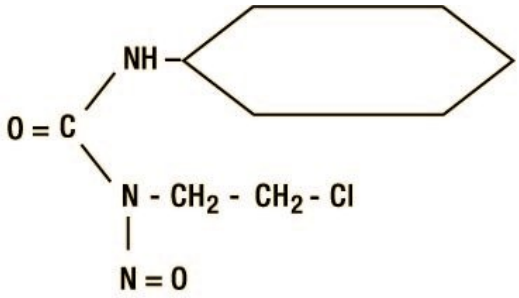
DESCRIPTION

Gleostine™ (lomustine) (CCNU) is one of the nitrosoureas used in the treatment of certain neoplastic diseases. It is 1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea. It is a yellow powder with the empirical formula of $C_9H_{16}ClN_3O_2$ and a molecular weight of 233.71. Gleostine is soluble in 10% ethanol (0.05 mg per mL) and in absolute alcohol (70 mg per mL). Gleostine is relatively insoluble in water (<0.05 mg per mL).

It is relatively un-ionized at a physiological pH.

Inactive ingredients in Gleostine Capsules are magnesium stearate and mannitol.

The structural formula is:



Gleostine is available in 10 mg, 40 mg, and 100 mg capsules for oral administration.

CLINICAL PHARMACOLOGY

Although it is generally agreed that lomustine alkylates DNA and RNA, it is not cross resistant with other alkylators. As with other nitrosoureas, it may also inhibit several key enzymatic processes by carbamoylation of amino acids in proteins.

Lomustine may be given orally. Following oral administration of radioactive lomustine at doses ranging from 30 mg/m^2 to 100 mg/m^2, about half of the radioactivity given was excreted in the urine in the form of degradation products within 24 hours.

The serum half-life of the metabolites ranges from 16 hours to 2 days. Tissue levels are comparable to plasma levels at 15 minutes after intravenous administration.

Because of the high lipid solubility and the relative lack of ionization at physiological pH, lomustine crosses the blood-brain barrier quite effectively. Levels of radioactivity in the CSF are 50% or greater than those measured concurrently in plasma.

INDICATIONS AND USAGE

Gleostine has been shown to be useful as a single agent in addition to other treatment modalities, or in established combination therapy with other approved chemotherapeutic agents in the following:

Brain tumors—both primary and metastatic, in patients who have already received appropriate surgical and/or radiotherapeutic procedures.

Hodgkin's disease—secondary therapy in combination with other approved drugs in patients who relapse while being treated with primary therapy, or who fail to respond to primary therapy.

CONTRAINDICATIONS

Gleostine should not be given to individuals who have demonstrated a previous hypersensitivity to it.

WARNINGS

Since the major toxicity is delayed bone marrow suppression, blood counts should be monitored weekly for at least 6 weeks after a dose (see ADVERSE REACTIONS). At the recommended dosage, courses of Gleostine should not be given more frequently than every 6 weeks.

The bone marrow toxicity of Gleostine is cumulative and therefore dosage adjustment must be considered on the basis of nadir blood counts from prior dose (see dosage adjustment table under **DOSAGE AND ADMINISTRATION**).

Pulmonary toxicity from Gleostine appears to be dose related (see **ADVERSE REACTIONS**).

Long-term use of nitrosoureas has been reported to be possibly associated with the development of secondary malignancies.

Liver and renal function tests should be monitored periodically (see **ADVERSE REACTIONS**).

Pregnancy Category D

Gleostine can cause fetal harm when administered to a pregnant woman. Lomustine is embryotoxic and teratogenic in rats and embryotoxic in rabbits at dose levels equivalent to the human dose. There are no adequate and well controlled studies in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking (receiving) this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

PRECAUTIONS

General

In all instances where the use of Gleostine is considered for chemotherapy, the physician must evaluate the need and usefulness of the drug against the risks of toxic effects or adverse reactions. Most such adverse reactions are reversible if detected early. When such effects or reactions do occur, the drug should be reduced in dosage or discontinued and appropriate corrective measures should be taken according to the clinical judgment of the physician. Reinstitution of Gleostine therapy should be carried out with caution and with adequate consideration of the further need for the drug and alertness as to possible recurrence of toxicity.

Information for Patients

Provide patients with the following information and instructions:

In order to provide the proper dose of Gleostine, the dose may be made up of 2 or more different strengths and colors of capsules. Each strength must be dispensed separately by the pharmacist.

1. Gleostine is given as a single oral dose and will not be repeated for at least 6 weeks. Daily use of the recommended dose may lead to toxicities and fatal outcomes.
2. Patients may experience nausea and vomiting that usually last less than 24 hours. Patients may also experience loss of appetite that may last for several days.
3. Instruct patients to contact their physician if they develop any of the following reactions: fever, chills, sore throat, unusual bleeding or bruising, shortness of breath, dry cough, swelling of feet or lower legs, mental confusion, or yellowing of eyes and skin.
4. Instruct patients to wear impervious (rubber or latex) gloves when handling Gleostine Capsules.

Laboratory Tests

Due to delayed bone marrow suppression, blood counts should be monitored weekly for at least 6 weeks after a dose.

Baseline pulmonary function studies should be conducted along with frequent pulmonary function tests during treatment. Patients with a baseline below 70% of the predicted Forced Vital Capacity (FVC) or Carbon Monoxide Diffusing Capacity (DL_{CO}) are particularly at risk.

Since Gleostine may cause liver dysfunction, it is recommended that liver function tests be monitored periodically.

Renal function tests should also be monitored periodically.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Lomustine is carcinogenic in rats and mice, producing a marked increase in tumor incidence in doses approximating those employed clinically. Nitrosourea therapy does have carcinogenic potential in humans (see **ADVERSE REACTIONS**). Lomustine also affects fertility in male rats at doses somewhat higher than the human dose.

Pregnancy

Pregnancy Category D

See **WARNINGS**.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Gleostine, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use

See **ADVERSE REACTIONS: Pulmonary Toxicity** and **DOSAGE AND ADMINISTRATION**.

Geriatric Use

No data from clinical studies of Gleostine are available for patients 65 years of age and over to determine whether they respond differently than younger patients. Other reported clinical experience has not identified differences in responses between elderly and younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function and of concomitant disease or other drug therapy.

Lomustine and its metabolites are known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, care should be taken in dose selection, and renal function should be monitored.

ADVERSE REACTIONS

Hematologic Toxicity

The most frequent and most serious toxicity of Gleostine is delayed myelosuppression. It usually occurs 4 to 6 weeks after drug administration and is dose related. Thrombocytopenia occurs at about 4 weeks postadministration and persists for 1 to 2 weeks. Leukopenia occurs at 5 to 6 weeks after a dose of Gleostine and persists for 1 to 2 weeks. Approximately 65% of patients receiving 130 mg/m^2 develop white blood counts below 5000 wbc/mm^3. Thirty-six percent developed white blood counts below 3000 wbc/mm^3. Thrombocytopenia is generally more severe than leukopenia. However, both may be dose-limiting toxicities.

Gleostine may produce cumulative myelosuppression, manifested by more depressed indices or longer duration of suppression after repeated doses.

The occurrence of acute leukemia and bone marrow dysplasias have been reported in patients following long-term nitrosourea therapy.

Anemia also occurs, but is less frequent and less severe than thrombocytopenia or leukopenia.

Pulmonary Toxicity

Pulmonary toxicity characterized by pulmonary infiltrates and/or fibrosis has been reported rarely with Gleostine. Onset of toxicity has occurred after an interval of 6 months or longer from the start of therapy with cumulative doses of Gleostine usually greater than 1100 mg/m^2. There is 1 report of pulmonary toxicity at a cumulative dose of only 600 mg.

Delayed onset pulmonary fibrosis occurring up to 17 years after treatment has been reported in patients who received related nitrosoureas in childhood and early adolescence (1–16 years) combined with cranial radiotherapy for intracranial tumors. There appeared to be some late reduction of pulmonary function of all long-term survivors. This form of lung fibrosis may be slowly progressive and has resulted in death in some cases. In this long-term study of carmustine, all those initially treated at less than 5 years of age died of delayed pulmonary fibrosis.

Gastrointestinal Toxicity

Nausea and vomiting may occur 3 to 6 hours after an oral dose and usually last less than 24 hours. Prior administration of antiemetics is effective in diminishing and sometimes preventing this side effect. Nausea and vomiting can also be reduced if Gleostine is administered to fasting patients.

Hepatotoxicity

A reversible type of hepatic toxicity, manifested by increased transaminase, alkaline phosphatase, and bilirubin levels, has been reported in a small percentage of patients receiving Gleostine.

Nephrotoxicity

Renal abnormalities consisting of progressive azotemia, decrease in kidney size, and renal failure have been reported in patients who received large cumulative doses after prolonged therapy with Gleostine. Kidney damage has also been reported occasionally in patients receiving lower total doses.

Other Toxicities

Stomatitis, alopecia, optic atrophy, and visual disturbances, such as blindness, have been reported infrequently.

Neurological reactions, such as disorientation, lethargy, ataxia, and dysarthria have been noted in some patients receiving Gleostine. However, the relationship to medication in these patients is unclear.

OVERDOSAGE

Accidental overdose with lomustine has been reported, including fatal cases. Accidental overdose has been associated with bone marrow suppression, abdominal pain, diarrhea, vomiting, anorexia, lethargy, dizziness, abnormal hepatic function, cough, and shortness of breath.

No proven antidotes have been established for Gleostine overdosage. In case of overdose, appropriate supportive measures should be taken.

DOSAGE AND ADMINISTRATION

The recommended dose of Gleostine in adult and pediatric patients as a single agent in previously untreated patients is 130 mg/m^2 as a single oral dose every 6 weeks (see **PRECAUTIONS: Information for Patients** and **HOW SUPPLIED: Directions to the Pharmacist**). In individuals with compromised bone marrow function, the dose should be reduced to 100 mg/m^2 every 6 weeks. When Gleostine is used in combination with other myelosuppressive drugs, the doses should be adjusted accordingly. All doses of Gleostine must be rounded to the nearest 10 mg by the prescriber (see **HOW SUPPLIED).**

Doses subsequent to the initial dose should be adjusted according to the hematologic response of the patient to the preceding dose. The following schedule is suggested as a guide to dosage adjustment:

Nadir After Prior Dose		**Percentage of Prior Dose to be Given**
Leukocytes (/mm^3)	**Platelets (/mm^3)**	
≥4000	≥100,000	100%
3000–3999	75,000–99,999	100%
2000–2999	25,000–74,999	70%
<2000	<25,000	50%

A repeat course of Gleostine should not be given until circulating blood elements have returned to acceptable levels (platelets above 100,000/mm^3; leukocytes above 4000/mm^3), and this is usually in 6 weeks. Adequate number of neutrophils should be present on a peripheral blood smear. Blood counts should be monitored weekly and repeat courses should not be given before 6 weeks because the hematologic toxicity is delayed and cumulative.

HOW SUPPLIED

Gleostine™ Capsules are available in individual bottles of 5 capsules each.

NDC 58181-3032-5	100 mg capsules (Green/Green)
NDC 58181-3031-5	40 mg capsules (White/Green)
NDC 58181-3030-5	10 mg capsules (White/White)

Stability

Gleostine Capsules are stable for the lot life indicated on package labeling when stored in well-closed containers at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature]. Avoid excessive heat (over 40°C, 104°F).

Directions to the Pharmacist

Confirm the total dose prescribed by the physician can be obtained by determining the appropriate combination of capsule strengths. Only the appropriate number of Gleostine capsules required for the administration of a single dose should be dispensed.

In order to provide the proper dose of Gleostine, patients should be aware that the prescribed dose may be made up of 2 or more different strengths and colors of capsules and that each strength must be dispensed separately. Inform patients that Gleostine is taken as a single oral dose and will not be repeated for at least 6 weeks. Daily use of the recommended dose may lead to toxicities and fatal outcomes.

Caution should be exercised when handling Gleostine Capsules. Procedures for proper handling and disposal of anticancer drugs should be utilized. Several guidelines on this subject have been published.[1-4] To minimize the risk of dermal exposure, always wear impervious gloves when handling bottles containing Gleostine Capsules. Gleostine Capsules should not be broken. Personnel should avoid exposure to broken capsules. If contact occurs, wash immediately and thoroughly. More information is available in the references listed below.

REFERENCES

1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165.
2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. Am J Health-Syst Pharm. 2006;63:1172–1193.

4. Polovich M, White JM, Kelleher LO, eds. 2005. Chemotherapy and biotherapy guidelines and recommendations for practice. 2nd ed. Pittsburgh, PA: Oncology Nursing Society.

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Manufactured by Corden Pharma Latina S.p.A., Sermoneta (LT), Italy for:
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Miami, FL 33155 USA

To report SUSPECTED ADVERSE REACTIONS, contact NextSource Biotechnology at 855-NSB-2468 (855-672-2468) or FDA at 1-800-FDA-1088 (1-800-332-1088) or www.fda.gov/medwatch.

Rev May 2014

Appendix 5. Response Assessment Using RECIST v.1.1 Criteria

Response Assessment Using RECIST Criteria

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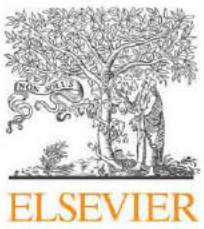

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New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1)

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ABSTRACT

Background: Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics: both tumour shrinkage (objective response) and disease progression are useful endpoints in clinical trials. Since RECIST was published in 2000, many investigators, cooperative groups, industry and government authorities have adopted these criteria in the assessment of treatment outcomes. However, a number of questions and issues have arisen which have led to the development of a revised RECIST guideline (version 1.1). Evidence for changes, summarised in separate papers in this special issue, has come from assessment of a large data warehouse (>6500 patients), simulation studies and literature reviews.

Highlights of revised RECIST 1.1: Major changes include: *Number of lesions to be assessed:* based on evidence from numerous trial databases merged into a data warehouse for analysis purposes, the number of lesions required to assess tumour burden for response determination has been reduced from a maximum of 10 to a maximum of five total (and from five to two per organ, maximum). *Assessment of pathological lymph* nodes is now incorporated: nodes with a *short* axis of $\geqslant$15 mm are considered measurable and assessable as target lesions. The short axis measurement should be included in the sum of lesions in calculation of tumour response. Nodes that shrink to <10 mm short axis are considered normal. *Confirmation of response* is required for trials with response primary endpoint but is no longer required in randomised studies since the control arm serves as appropriate means of interpretation of data. *Disease progression* is clarified in several aspects: in addition to the previous definition of progression in target disease of 20% increase in sum, a 5 mm absolute increase is now required as well to guard against over calling PD when the total sum is very

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small. Furthermore, there is guidance offered on what constitutes 'unequivocal progres sion' of non measurable/non target disease, a source of confusion in the original RECIST guideline. Finally, a section on detection of new lesions, including the interpretation of FDG PET scan assessment is included. *Imaging guidance*: the revised RECIST includes a new imaging appendix with updated recommendations on the optimal anatomical assess ment of lesions.

Future work: A key question considered by the RECIST Working Group in developing RECIST 1.1 was whether it was appropriate to move from anatomic unidimensional assessment of tumour burden to either volumetric anatomical assessment or to functional assessment with PET or MRI. It was concluded that, at present, there is not sufficient standardisation or evidence to abandon anatomical assessment of tumour burden. The only exception to this is in the use of FDG PET imaging as an adjunct to determination of progression. As is detailed in the final paper in this special issue, the use of these promising newer approaches requires appropriate clinical validation studies.

1. Background

1.1. History of RECIST criteria

Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics. Both tumour shrinkage (objective response) and time to the devel opment of disease progression are important endpoints in cancer clinical trials. The use of tumour regression as the endpoint for phase II trials screening new agents for evi dence of anti tumour effect is supported by years of evi dence suggesting that, for many solid tumours, agents which produce tumour shrinkage in a proportion of patients have a reasonable (albeit imperfect) chance of subsequently demonstrating an improvement in overall survival or other time to event measures in randomised phase III studies (re viewed in [1 4]). At the current time objective response car ries with it a body of evidence greater than for any other biomarker supporting its utility as a measure of promising treatment effect in phase II screening trials. Furthermore, at both the phase II and phase III stage of drug development, clinical trials in advanced disease settings are increasingly utilising time to progression (or progression free survival) as an endpoint upon which efficacy conclusions are drawn, which is also based on anatomical measurement of tumour size.

However, both of these tumour endpoints, objective re sponse and time to disease progression, are useful only if based on widely accepted and readily applied standard crite ria based on anatomical tumour burden. In 1981 the World Health Organisation (WHO) first published tumour response criteria, mainly for use in trials where tumour response was the primary endpoint. The WHO criteria introduced the con cept of an overall assessment of tumour burden by summing the products of bidimensional lesion measurements and determined response to therapy by evaluation of change from baseline while on treatment.[5] However, in the decades that followed their publication, cooperative groups and pharma ceutical companies that used the WHO criteria often 'modi fied' them to accommodate new technologies or to address areas that were unclear in the original document. This led to confusion in interpretation of trial results[6] and in fact, the application of varying response criteria was shown to lead to very different conclusions about the efficacy of the same regimen.[7] In response to these problems, an International Working Party was formed in the mid 1990s to standardise and simplify response criteria. New criteria, known as RECIST (Response Evaluation Criteria in Solid Tumours), were pub lished in 2000.[8] Key features of the original RECIST include definitions of minimum size of measurable lesions, instruc tions on how many lesions to follow (up to 10; a maximum five per organ site), and the use of unidimensional, rather than bidimensional, measures for overall evaluation of tu mour burden. These criteria have subsequently been widely adopted by academic institutions, cooperative groups, and industry for trials where the primary endpoints are objective response or progression. In addition, regulatory authorities accept RECIST as an appropriate guideline for these assessments.

1.2. Why update RECIST?

Since RECIST was published in 2000, many investigators have confirmed in prospective analyses the validity of substituting unidimensional for bidimensional (and even three dimen sional) based criteria (reviewed in [9]). With rare exceptions (e.g. mesothelioma), the use of unidimensional criteria seems to perform well in solid tumour phase II studies.

However, a number of questions and issues have arisen which merit answers and further clarity. Amongst these are whether fewer than 10 lesions can be assessed without affecting the overall assigned response for patients (or the conclusion about activity in trials); how to apply RECIST in randomised phase III trials where progression, not response, is the primary endpoint particularly if not all patients have measurable disease; whether or how to utilise newer imag ing technologies such as FDG PET and MRI; how to handle assessment of lymph nodes; whether response confirmation is truly needed; and, not least, the applicability of RECIST in trials of targeted non cytotoxic drugs. This revision of the RECIST guidelines includes updates that touch on all these points.

1.3. *Process of RECIST 1.1 development*

The RECIST Working Group, consisting of clinicians with expertise in early drug development from academic research organisations, government and industry, together with imaging specialists and statisticians, has met regularly to set the agenda for an update to RECIST, determine the evidence needed to justify the various changes made, and to review emerging evidence. A critical aspect of the revision process was to create a database of prospectively documented solid tumour measurement data obtained from industry and academic group trials. This database, assembled at the EORTC Data Centre under the leadership of Jan Bogaerts and Patrick Therasse (co authors of this guideline), consists of >6500 patients with >18,000 target lesions and was utilised to investigate the impact of a variety of questions (e.g. number of target lesions required, the need for response confirmation, and lymph node measurement rules) on response and progression free survival outcomes. The results of this work, which after evaluation by the RECIST Working Group led to most of the changes in this revised guideline, are reported in detail in a separate paper in this special issue.[10] Larry Schwartz and Robert Ford (also co authors of this guideline) also provided key databases from which inferences have been made that inform these revisions.[11]

The publication of this revised guideline is believed to be timely since it incorporates changes to simplify, optimise and standardise the assessment of tumour burden in clinical trials. A summary of key changes is found in Appendix I. Because the fundamental approach to assessment remains grounded in the anatomical, rather than functional, assessment of disease, we have elected to name this version RECIST 1.1, rather than 2.0.

1.4. *What about volumetric or functional assessment?*

This raises the question, frequently posed, about whether it is 'time' to move from anatomic unidimensional assessment of tumour burden to either volumetric anatomical assessment *or* to functional assessment (e.g. dynamic contrast enhanced MRI or CT or (18)F fluorodeoxyglucose positron emission tomographic (FDG PET) techniques assessing tumour metabolism). As can be seen, the Working Group and particularly those involved in imaging research, did not believe that there is at present sufficient standardisation and widespread availability to recommend adoption of these alternative assessment methods. The only exception to this is in the use of FDG PET imaging as an adjunct to determination of progression, as described later in this guideline. As detailed in paper in this special issue[12], we believe that the use of these promising newer approaches (which could either *add to* or *substitute for* anatomical assessment as described in RECIST) requires appropriate and rigorous clinical validation studies. This paper by Sargent et al. illustrates the type of data that will be needed to be able to define 'endpoints' for these modalities and how to determine where and when such criteria/modalities can be used to improve the reliability with which truly active new agents are identified and truly inactive new agents are discarded in comparison to RECIST criteria in phase II screening trials. The RECIST Working Group looks forward to such data emerging in the next few years to allow the appropriate changes to the next iteration of the RECIST criteria.

2. Purpose of this guideline

This guideline describes a standard approach to solid tumour measurement and definitions for objective assessment of change in tumour size for use in adult and paediatric cancer clinical trials. It is expected these criteria will be useful in all trials where objective response is the primary study endpoint, as well as in trials where assessment of stable disease, tumour progression or time to progression analyses are undertaken, since all of these outcome measures are based on an assessment of anatomical tumour burden and its change on study. There are no assumptions in this paper about the proportion of patients meeting the criteria for any of these endpoints which will signal that an agent or treatment regimen is active: those definitions are dependent on type of cancer in which a trial is being undertaken and the specific agent(s) under study. Protocols must include appropriate statistical sections which define the efficacy parameters upon which the trial sample size and decision criteria are based. In addition to providing definitions and criteria for assessment of tumour response, this guideline also makes recommendations regarding standard reporting of the results of trials that utilise tumour response as an endpoint.

While these guidelines may be applied in malignant brain tumour studies, there are also separate criteria published for response assessment in that setting.[13] This guideline is *not* intended for use for studies of malignant lymphoma since international guidelines for response assessment in lymphoma are published separately.[14]

Finally, many oncologists in their daily clinical practice follow their patients' malignant disease by means of repeated imaging studies and make decisions about continued therapy on the basis of both objective and symptomatic criteria. It is not intended that these RECIST guidelines play a role in that decision making, except if determined appropriate by the treating oncologist.

3. Measurability of tumour at baseline

3.1. *Definitions*

At baseline, tumour lesions/lymph nodes will be categorised measurable or non measurable as follows:

3.1.1. *Measurable*

Tumour lesions: Must be accurately measured in at least one dimension (*longest* diameter in the plane of measurement is to be recorded) with a *minimum* size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm; see Appendix II on imaging guidance).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non measurable).
- 20 mm by chest X ray.

Malignant lymph nodes: To be considered pathologically en larged *and* measurable, a lymph node must be ⩾15 mm in *short* axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow up, only the *short* axis will be measured and followed (see Schwartz et al. in this Special Issue[15]). See also notes be low on 'Baseline documentation of target and non target le sions' for information on lymph node measurement.

3.1.2. *Non-measurable*

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ⩾10 to <15 mm short axis) as well as truly non measurable lesions. Lesions considered truly non measurable include: leptomeningeal dis ease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

3.1.3. *Special considerations regarding lesion measurability*

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:.

- Bone scan, PET scan or plain films are not considered ade quate imaging techniques to measure bone lesions. How ever, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic blastic lesions, with *identi fiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be con sidered as measurable lesions if the *soft tissue component* meets the definition of measurability described above.
- Blastic bone lesions are non measurable.

Cystic lesions:.

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the defi nition of measurability described above. However, if non cystic lesions are present in the same patient, these are pre ferred for selection as target lesions.

Lesions with prior local treatment:

- Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco regional therapy, are usu ally not considered measurable unless there has been dem onstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

3.2. *Specifications by methods of measurements*

3.2.1. *Measurement of lesions*

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

3.2.2. *Method of assessment*

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow up. Imaging based evalu ation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered mea surable when they are superficial and ⩾10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography including a ru ler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X ray: Chest CT is preferred over chest X ray, particu larly when progression is an important endpoint, since CT is more sensitive than X ray, particularly in identifying new le sions. However, lesions on chest X ray may be considered measurable if they are clearly defined and surrounded by aer ated lung. See Appendix II for more details.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. As is described in Appendix II, when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). More details concerning the use of both CT and MRI for assessment of objective tumour response evaluation are provided in Appendix II.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their en tirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is ad vised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilisation of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumour markers: Tumour markers *alone* cannot be used to as sess *objective* tumour response. If markers are initially above

the upper normal limit, however, they must normalise for a patient to be considered in complete response. Because tumour markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for both CA 125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer), have been published.[16–18] In addition, the Gynecologic Cancer Intergroup has developed CA125 progression criteria which are to be integrated with objective tumour assessment for use in first line trials in ovarian cancer.[19]

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumour types such as germ cell tumours, where known residual benign tumours can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumour has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

4. Tumour response evaluation

4.1. *Assessment of overall tumour burden and measurable disease*

To assess objective response or future progression, it is necessary to estimate the *overall tumour burden at baseline* and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 3). In studies where the primary endpoint is tumour progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non measurable disease only are also eligible.

4.2. *Baseline documentation of 'target' and 'non-target' lesions*

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as *target lesions* and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a *maximum* of two and four lesions respectively will be recorded). For evidence to support the selection of only five target lesions, see analyses on a large prospective database in the article by Bogaerts et al.[10].

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to *reproducible repeated measurements*. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. To illustrate this point see the example in Fig. 3 of Appendix II.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumour. As noted in Section 3, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of $\geqslant$15 mm by CT scan. Only the *short* axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumour. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm $\times$ 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement (See also the example in Fig. 4 in Appendix II). All other pathological nodes (those with short axis $\geqslant$10 mm but <15 mm) should be considered non target lesions. Nodes that have a short axis <10 mm are considered non pathological and should not be recorded or followed.

A *sum of the diameters* (longest for non nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the *baseline sum diameters*. If lymph nodes are to be included in the sum, then as noted above, only the *short* axis is added into the sum. The baseline sum diameters will be used as reference to further characterise any objective tumour regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as *non target lesions* and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

4.3. *Response criteria*

This section provides the definitions of the criteria used to determine objective tumour response for target lesions.

4.3.1. *Evaluation of target lesions*

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the *smallest sum on study* (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (*Note:* the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

4.3.2. *Special notes on the assessment of target lesions*

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (*Note:* It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that split or coalesce on treatment. As noted in Appendix II, when non nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

4.3.3. *Evaluation of non-target lesions*

This section provides the definitions of the criteria used to determine the tumour response for the group of non target lesions. While some non target lesions may actually be measurable, they need not be measured and instead should be assessed only *qualitatively* at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non target lesions and normalisation of tumour marker level. All lymph nodes must be non pathological in size (<10 mm short axis).

Non CR/Non PD: Persistence of one or more non target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): *Unequivocal progression* (see comments below) of existing non target lesions. (*Note:* the appearance of one or more new lesions is also considered progression).

4.3.4. *Special notes on assessment of progression of non-target disease*

The concept of progression of non target disease requires additional explanation as follows:

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non target disease, there must be an overall level of substantial worsening in non target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy (see examples in Appendix II and further details below). A modest 'increase' in the size of one or more non target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non measurable disease burden. Because worsening in non target disease cannot be easily quantified (by definition: if all lesions are truly non measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e. an increase in tumour burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic

disease from localised to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. Some illustrative examples are shown in Figs. 5 and 6 in Appendix II. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

4.3.5. *New lesions*

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some 'new' bone lesions may be simply healing or flare of pre existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow up study in an anatomical location that was *not* scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG PET imaging can be identified according to the following algorithm:

a. Negative FDG PET at baseline, with a positive[1] FDG PET at follow up is a sign of PD based on a new lesion.
b. No FDG PET at baseline and a positive FDG PET at follow up:
   If the positive FDG PET at follow up corresponds to a new site of disease confirmed by CT, this is PD.
   If the positive FDG PET at follow up is not confirmed as a new site of disease on CT, additional follow up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG PET scan).
   If the positive FDG PET at follow up corresponds to a pre existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

[1] A 'positive' FDG PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

4.4. *Evaluation of best overall response*

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement (see Section 4.6). Specifically, in non randomised trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

4.4.1. *Time point response*

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 on the next page provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

When patients have non measurable (therefore non target) disease only, Table 2 is to be used.

4.4.2. *Missing assessments and inevaluable designation*

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

4.4.3. *Best overall response: all time points*

The *best overall response* is determined once all the data for the patient is known.

Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is other wise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow up after the first SD assessment would be considered inevaluable.

Table 1 – Time point response: patients with target (+/– non-target) disease.

Target lesions	Non target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non CR/non PD	No	PR
CR	Not evaluated	No	PR
PR	Non PD or not all evaluated	No	PR
SD	Non PD or not all evaluated	No	SD
Not all evaluated	Non PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2 – Time point response: patients with non-target disease only.

Non target lesions	New lesions	Overall response
CR	No	CR
Non CR/non PD	No	Non CR/non PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non CR/non PD' is preferred over 'stable disease' for non target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Best response determination in trials where confirmation of complete or partial response IS required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3.

4.4.4. *Special notes on response assessment*

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

In trials where confirmation of response is required, repeated 'NE' time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR NE PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is *not* a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non target disease as shown in Tables 1 3.

Conditions that define 'early progression, early death and inevaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

a If a CR is *truly* met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

needle aspirate/biopsy) before assigning a status of complete response. FDG PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG PET in this circumstance should be prospectively described in the protocol and supported by dis ease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG PET and biopsy res olution/sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

4.5. *Frequency of tumour re-evaluation*

Frequency of tumour re evaluation while on treatment should be protocol specific and adapted to the type and sche dule of treatment. However, in the context of phase II studies where the beneficial effect of therapy is not known, follow up every 6 8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances. The proto col should specify which organ sites are to be evaluated at baseline (usually those most likely to be involved with meta static disease for the tumour type under study) and how often evaluations are repeated. Normally, all target and non target sites are evaluated at each assessment. In selected circum stances certain non target organs may be evaluated less fre quently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

After the end of the treatment, the need for repetitive tu mour evaluations depends on whether the trial has as a goal the response rate or the time to an event (progression/death). If 'time to an event' (e.g. time to progression, disease free survival, progression free survival) is the main endpoint of the study, then routine scheduled re evaluation of protocol specified sites of disease is warranted. In randomised com parative trials in particular, the scheduled assessments should be performed as identified on a calendar schedule (for example: every 6 8 weeks on treatment or every 3 4 months after treatment) and should not be affected by delays in therapy, drug holidays or any other events that might lead to imbalance in a treatment arm in the timing of disease assessment.

4.6. *Confirmatory measurement/duration of response*

4.6.1. *Confirmation*

In non randomised trials where response is the primary end point, confirmation of PR and CR is required to ensure re sponses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see the paper by Bogaerts et al. in this Special Issue[10]). However, in all other circum stances, i.e. in randomised trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add va lue to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in partic ular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6 8 weeks) that is defined in the study protocol.

4.6.2. *Duration of overall response*

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive dis ease is objectively documented (taking as reference for progres sive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

4.6.3. *Duration of stable disease*

Stable disease is measured from the start of the treatment (in randomised trials, from date of randomisation) until the crite ria for progression are met, taking as reference the *smallest sum on study* (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease var ies in different studies and diseases. If the proportion of pa tients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and stable disease as well as the progression free survival are influenced by the frequency of follow up after baseline evaluation. It is not in the scope of this guideline to define a standard follow up frequency. The fre quency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if compari sons between trials are to be made.

4.7. *Progression-free survival/proportion progression-free*

4.7.1. *Phase II trials*

This guideline is focused primarily on the use of objective re sponse endpoints for phase II trials. In some circumstances, 're sponse rate' may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases 'progression free survival' (PFS) or the 'proportion pro gression free' at landmark time points, might be considered appropriate alternatives to provide an initial signal of biologic effect of new agents. It is clear, however, that in an uncontrolled trial, these measures are subject to criticism since an appar ently promising observation may be related to biological factors such as patient selection and not the impact of the intervention. Thus, phase II screening trials utilising these endpoints are best designed with a randomised control. Exceptions may exist

where the behaviour patterns of certain cancers are so consis tent (and usually consistently poor), that a non randomised trial is justifiable (see for example van Glabbeke et al.[20]). How ever, in these cases it will be essential to document with care the basis for estimating the expected PFS or proportion progres sion free in the absence of a treatment effect.

4.7.2. *Phase III trials*

Phase III trials in advanced cancers are increasingly designed to evaluate progression free survival or time to progression as the primary outcome of interest. Assessment of progression is relatively straightforward if the protocol requires all pa tients to have measurable disease. However, restricting entry to this subset of patients is subject to criticism: it may result in a trial where the results are less likely to be generalisable if, in the disease under study, a substantial proportion of pa tients would be excluded. Moreover, the restriction to entry will slow recruitment to the study. Increasingly, therefore, tri als allow entry of both patients with measurable disease as well as those with non measurable disease only. In this cir cumstance, care must be taken to explicitly describe the find ings which would qualify for progressive disease for those patients *without* measurable lesions. Furthermore, in this set ting, protocols must indicate if the maximum number of re corded target lesions for those patients with measurable disease may be relaxed from five to three (based on the data found in Bogaerts et al.[10] and Moskowitz et al.[11]). As found in the 'special notes on assessment of progression', these guide lines offer recommendations for assessment of progression in this setting. Furthermore, if available, validated tumour mar ker measures of progression (as has been proposed for ovarian cancer) may be useful to integrate into the definition of pro gression. Centralised blinded review of imaging studies or of source imaging reports to verify 'unequivocal progression' may be needed if important drug development or drug ap proval decisions are to be based on the study outcome. Finally, as noted earlier, because the date of progression is subject to ascertainment bias, timing of investigations in study arms should be the same. The article by Dancey et al. in this special issue[21] provides a more detailed discussion of the assessment of progression in randomised trials.

4.8. *Independent review of response and progression*

For trials where *objective response* (CR + PR) is the primary end point, and in particular where key drug development deci sions are based on the observation of a minimum number of responders, it is recommended that all claimed responses be reviewed by an expert(s) independent of the study. If the study is a randomised trial, ideally reviewers should be blinded to treatment assignment. Simultaneous review of the patients' files and radiological images is the best approach.

Independent review of progression presents some more complex issues: for example, there are statistical problems with the use of central review based progression time in place of investigator based progression time due to the poten tial introduction of informative censoring when the former precedes the latter. An overview of these factors and other lessons learned from independent review is provided in an article by Ford et al. in this special issue.[22]

4.9. *Reporting best response results*

4.9.1. *Phase II trials*

When response is the primary endpoint, and thus all patients must have measurable disease to enter the trial, all patients included in the study must be accounted for in the report of the results, even if there are major protocol treatment devia tions or if they are not evaluable. Each patient will be assigned one of the following categories:

1. Complete response
2. Partial response
3. Stable disease
4. Progression
5. Inevaluable for response: specify reasons (for example: early death, malignant disease; early death, toxicity; tumour assessments not repeated/incomplete; other (specify)).

Normally, all *eligible* patients should be included in the denominator for the calculation of the response rate for phase II trials (in some protocols it will be appropriate to include all treated patients). It is generally preferred that 95% two sided confidence limits are given for the calculated response rate. Trial conclusions should be based on the response rate for all eligible (or all treated) patients and should *not* be based on a selected 'evaluable' subset.

4.9.2. *Phase III trials*

Response evaluation in phase III trials may be an indicator of the relative anti tumour activity of the treatments eval uated and is almost always a secondary endpoint. Ob served differences in response rate may not predict the clinically relevant therapeutic benefit for the population studied. If objective response is selected as a primary end point for a phase III study (only in circumstances where a direct relationship between objective tumour response and a clinically relevant therapeutic benefit can be unambigu ously demonstrated for the population studied), the same criteria as those applying to phase II trials should be used and all patients entered should have at least one measur able lesion.

In those many cases where response is a secondary end point and not all trial patients have measurable disease, the method for reporting overall best response rates must be pre specified in the protocol. In practice, response rate may be reported using either an 'intent to treat' analysis (all ran domised patients in the denominator) or an analysis where only the subset of patients with measurable disease at baseline are included. The protocol should clearly specify how response results will be reported, including any subset analyses that are planned.

The original version of RECIST suggested that in phase III trials one could write protocols using a 'relaxed' interpreta tion of the RECIST guidelines (for example, reducing the num ber of lesions measured) but this should no longer be done since these revised guidelines have been amended in such a way that it is clear how these criteria should be applied for all trials in which anatomical assessment of tumour response or progression are endpoints.

Appendix I. Summary of major changes RECIST 1.0 to RECIST 1.1

	RECIST 1.0	RECIST 1.1	Rationale	Reference in special issue (if applicable)
Minimum size measurable lesions	CT: 10 mm spiral 20 mm non spiral	CT 10 mm; delete reference to spiral scan	Most scans used have 5 mm or less slice thickness Clearer to give instruction based on slice interval if it is greater than 5 mm	
	Clinical: 20 mm	Clinical: 10 mm (must be measurable with calipers)	Caliper measurement will make this reliable	
	Lymph node: not mentioned	CT: ⩾15 mm short axis for target ⩾10 <15 mm for non target <10 mm is non pathological	Since nodes are normal structure need to define pathological enlargement. Short axis is most sensitive	Schwartz et al.[15]
Special considerations on lesion measurability		Notes included on bone lesions, cystic lesions	Clarify frequently asked questions	
Overall tumour burden	10 lesions (5 per organ)	5 lesions (2 per organ)	Data warehouse analysis shows no loss of information if lesion number reduced from 10 to 5. A maximum of 2 lesions per organ yields sufficient representation per disease site	Bogaerts et al.[10]
Response criteria target disease	CR lymph node not mentioned	CR lymph nodes must be <10 mm short axis	In keeping with normal size of nodes	Schwartz et al.[15]
	PD 20% increase over smallest sum on study or new lesions	PD 20% increase over smallest sum on study (including baseline if that is smallest) and at least 5 mm increase or new lesions	Clarification that if baseline measurement is smaller than any on study measurement, it is reference against which PD is assessed 5 mm absolute increase to guard against over calling PD when total sum is very small and 20% increase is within measurement error	
Response criteria non target disease	'unequivocal progression' considered as PD	More detailed description of 'unequivocal progression' to indicate that it should not normally trump target disease status. It must be representative of overall disease status change, not a single lesion increase	Confusion with RECIST 1.0 where some were considering PD if 'increase' in any non target lesion, even when target disease is stable or responding	
New lesions		New section on New lesions	To provide guidance on when a lesion is considered new (and thus PD)	
Overall response	Table integrated target and non target lesions	Two tables: one integrating target and non target and the other of non target only	To account for the fact that RECIST criteria are now being used in trials where PFS is the endpoint and not all patients have measurable (target) disease at baseline	Dancey et al.[21]

	RECIST 1.0	RECIST 1.1	Rationale	Reference in special issue (if applicable)
		Special notes: How to assess and measure lymph nodes CR in face of residual tissue Discussion of 'equivocal' progression	Frequently asked questions on these topics	
Confirmatory measure	For CR and PR: criteria must be met again 4 weeks after initial documentation	Retain this requirement ONLY for non randomised trials with primary endpoint of response	Data warehouse shows that response rates rise when confirmation is eliminated, but the only circumstance where this is important is in trials where there is no concurrent comparative control and where this measure is the primary endpoint	Bogaerts et al.[10]
Progression free survival	General comments only	More specific comments on use of PFS (or proportion progression free) as phase II endpoint Greater detail on PFS assessment in phase III trials	Increasing use of PFS in phase III trials requires guidance on assessment of PD in patients with non measurable disease	Dancey et al.[21]
Reporting of response results	9 categories suggested for reporting phase II results	Divided into phase II and phase III 9 categories collapsed into 5 In phase III, guidance given about reporting response	Simplifies reporting and clarifies how to report phase II and III data consistently	
Response in phase III trials	More relaxed guidelines possible if protocol specified	This section removed and referenced in section above: no need to have different criteria for phase II and III	Simplification of response assessment by reducing number of lesions and eliminating need for confirmation in randomised studies where response is not the primary endpoint makes separate 'rules' unnecessary	
Imaging appendix	Appendix I	Appendix II: updated with detailed guidance on use of MRI, PET/CT Other practical guidance included	Evolving use of newer modalities addressed. Enhanced guidance in response to frequent questions and from radiology review experience	
New appendices		Appendix I: comparison of RECIST 1.0 and 1.1 Appendix III: frequently asked questions		

Conflict of interest statement

None declared.

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Appendix II. Specifications for standard anatomical radiological imaging

These protocols for image acquisition of computed tomography (CT) and magnetic resonance imaging (MRI) are recommendations intended for patients on clinical trials where RECIST assessment will be performed. Standardisation of imaging requirements and image acquisition parameters is ideal to allow for optimal comparability of subjects within a study and results between studies. These recommendations are designed to balance optimised image acquisition protocols with techniques that should be feasible to perform globally at imaging facilities in all types of radiology practices. These guidelines are not applicable to functional imaging techniques or volumetric assessment of tumour size.

Scanner quality control is highly recommended and should follow standard manufacturer and facility maintenance schedules using commercial phantoms. It is likely that for RECIST unidimensional measurements this will be adequate to produce reproducible measurements. Imaging quality control for CT includes an analysis of image noise and uniformity and CT number as well as spatial resolution. The frequency of quality control analysis is also variable and should focus on clinically relevant scanning parameters. Dose analysis is always important and the use of imaging should follow the ALARA principle, 'As Low As Reasonably Achievable', which refers to making every reasonable effort to maintain radiation exposures as far below the dose limits as possible.

Specific notes

Chest X ray measurement of lesions surrounded by pulmonary parenchyma is feasible, but not preferable as the measurement represents a summation of densities. Furthermore, there is poor identification of new lesions within the chest on X ray as compared with CT. Therefore, measurements of pulmonary parenchymal lesions as well as mediastinal disease are optimally performed with CT of the chest. MRI of the chest should only be performed in extenuating circumstances. Even if IV contrast cannot be administered (for example, in the situation of allergy to contrast), a non contrast CT of the chest is still preferred over MRI or chest X ray.

CT scans: CT scans of the chest, abdomen, and pelvis should be contiguous throughout all the anatomic region of interest. As a general rule, the minimum size of a measurable lesion at baseline should be no less than double the slice thickness and also have a minimum size of 10 mm (see below for minimum size when scanners have a slice thickness more than 5 mm). While the precise physics of lesion size and partial volume averaging is complex, lesions smaller than 10 mm may be difficult to accurately and reproducibly measure. While this rule is applicable to baseline scans, as lesions potentially decrease in size at follow up CT studies, they should still be measured. Lesions which are reported as 'too small to measure' should be assigned a default measurement of 5 mm if they are still visible.

The most critical CT image acquisition parameters for optimal tumour evaluation using RECIST are *anatomic coverage, contrast administration, slice thickness, and reconstruction interval.*

a. *Anatomic coverage*: Optimal anatomic coverage for most solid tumours is the chest, abdomen and pelvis. Coverage should encompass all areas of known predilection for metastases in the disease under evaluation and

should additionally investigate areas that may be involved based on signs and symptoms of individual patients. Because a lesion later identified in a body part not scanned at baseline would be considered as a new lesion representing disease progression, careful consid eration should be given to the extent of imaging coverage at baseline and at subsequent follow up time points. This will enable better consistency not only of tumour measurements but also identification of new disease.

b. *IV contrast administration*: Optimal visualisation and measurement of metastases in solid tumours requires consistent administration (dose and rate) of IV contrast as well as timing of scanning. Typically, most abdomi nal imaging is performed during the portal venous phase and (optimally) about the same time frame after injection on each examination (see Fig. 1 for impact of different phase of IV contrast on lesion measurement). Most solid tumours may be scanned with a single phase after administration of contrast. While triphasic CT scans are sometimes performed on other types of vascular tumours to improve lesion conspicuity, for consistency and uniformity, we would recommend tri phasic CT for hepatocellular and neuroendocrine tumours for which this scanning protocol is generally standard of care, and the improved temporal resolution of the triphasic scan will enhance the radiologists' abil ity to consistently and reproducibly measure these lesions. The precise dose and rate of IV contrast is dependent upon the CT scanning equipment, CT acqui sition protocol, the type of contrast used, the available venous access and the medical condition of the patient. Therefore, the method of administration of intravenous contrast agents is variable. Rather than try to institute rigid rules regarding methods for administering contrast agents and the volume injected, it is appropriate to suggest that an adequate volume of a suitable contrast agent should be given so that the metastases are demonstrated to best effect and a *con sistent method* is used on subsequent examinations for any given patient (ideally, this would be specified in the protocol or for an institution). It is very important that the same technique be used at baseline and on fol low up examinations for a given patient. This will greatly enhance the reproducibility of the tumour mea surements. If prior to enrolment it is known a patient is not able to undergo CT scans with IV contrast due to allergy or renal insufficiency, the decision as to whether a non contrast CT or MRI (with or without IV contrast) should be used to evaluate the subject at baseline and follow up should be guided by the tumour type under investigation and the anatomic location of the disease. For patients who develop contraindica tions to contrast after baseline contrast CT is done, the decision as to whether non contrast CT or MRI (enhanced or non enhanced) should be performed should also be based on the tumour type, anatomic location of the disease and should be optimised to allow for comparison to the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions on a different modality and interpretation of non target disease or new lesions, since the same lesion may appear to have a different size using a new modality (see Fig. 2 for a comparison of CT and MRI of the same lesion). Oral contrast is recommended to help visualise and differ entiate structures in the abdomen.

c. *Slice thickness and reconstruction interval*: RECIST measure ments may be performed at most clinically obtained slice thicknesses. It is recommended that CT scans be performed at 5 mm contiguous slice thickness or less and indeed this guideline presumes a minimum 5 mm thickness in recommendations for measurable lesion definition. Indeed, variations in slice thickness can have an impact on lesion measurement and on detection of new lesions. However, consideration should also be given for minimising radiation exposure. With these parameters, a minimum 10 mm lesion is considered measurable at baseline. Occasionally, institutions may perform medically acceptable scans at slice thicknesses *greater than* 5 mm. If this occurs, the minimum size of measurable lesions at baseline should be *twice the slice*

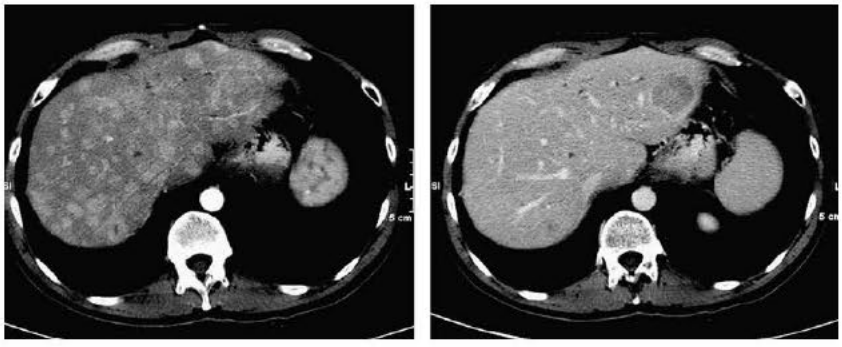

Fig. 1 – Difference in measurement/visualisation with different phases of IV contrast administration. Hypervascular metastases imaged in the arterial phase (left) and the portal venous phase (right). Note that the number of lesions visible differs greatly between the two phases of contrast administration as does any potential lesion measurement. Consistent CT scan acquisition, including phase of contrast administration, is important for optimal and reproducible tumour

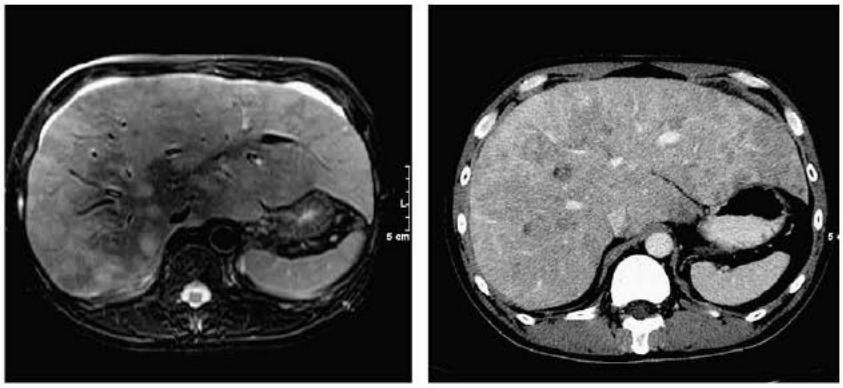

Fig. 2 – CT versus MRI of same lesions showing apparent 'progression' due only to differing method of measurement.

thickness of the baseline scans. Most contemporary CT scanners are multidetector which have many imaging options for these acquisition parameters.[23] The equip ment vendor and scanning manual should be reviewed if there are any specific system questions.

d. *Alternative contrast agents*: There are a number of other, new contrast agents, some organ specific.[24] They may be used as part of patient care for instance, in liver lesion assessment, or lymph node characterisation[25], but *should not as yet be used in clinical trials*.

FDG PET has gained acceptance as a valuable tool for detecting, staging and restaging several malignancies. Criteria for incorporating (or substituting) FDG PET into anatomical assessment of tumour response in phase II trials are not yet available, though much research is ongoing. Nevertheless, FDG PET is being used in many drug development trials both as a tool to assess therapeutic efficacy and also in assessment of progression. If FDG PET scans are included in a protocol, by consensus, an FDG uptake period of 60 min prior to imaging has been decided as the most appropriate for imaging of pa tients with malignancy.[26] Whole body acquisition is impor tant since this allows for sampling of all areas of interest and can assess if new lesions have appeared thus determining the possibility of interval progression of disease. Images from the base of the skull to the level of the mid thigh should be ob tained 60 min post injection. PET camera specifications are variable and manufacturer specific, so every attempt should be made to use the same scanner, or the same model scanner, for serial scans on the same patient. Whole body acquisitions can be performed in either 2 or 3 dimensional mode with attenuation correction, but the method chosen should be con sistent across all patients and serial scans in the clinical trial.

PET/CT scans: Combined modality scanning such as with PET CT is increasingly used in clinical care, and is a modal ity/technology that is in rapid evolution; therefore, the recom mendations in this paper may change rather quickly with time. At present, low dose or attenuation correction CT por tions of a combined PET CT are of limited use in anatomically based efficacy assessments and it is therefore suggested that they should not be substituted for dedicated diagnostic con trast enhanced CT scans for anatomically based RECIST mea surements. However, if a site can document that the CT performed as part of a PET CT is of identical diagnostic qual ity to a diagnostic CT (with IV and oral contrast) then the CT portion of the PET CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces addi tional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound examinations should not be used in clinical trials to measure tumour regression or progression of lesions be cause the examination is necessarily subjective and operator dependent. The reasons for this are several: Entire examina tions cannot be reproduced for independent review at a later date, and it must be assumed, whether or not it is the case, that the hard copy films available represent a true and accu rate reflection of events. Furthermore, if, for example, the only measurable lesion is in the para aortic region of the abdomen and if gas in the bowel overlies the lesion, the lesion will not be detected because the ultrasound beam cannot penetrate the gas. Accordingly, the disease staging (or restag ing for treatment evaluation) for this patient will not be accurate.

While evaluation of lesions by *physical examination* is also of limited reproducibility, it is permitted when lesions are superficial, at least 10 mm size, and can be assessed using calipers. In general, it is preferred if patients on clinical trials have at least one lesion that is measurable by CT. Other skin or palpable lesions may be measured on physical examina tion and be considered target lesions.

Use of *MRI* remains a complex issue. MRI has excellent contrast, spatial and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity and mea surement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimised for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. Generally, axial imaging of the abdomen and pelvis with T1 and T2 weighted imaging along with gadolinium enhanced imaging should be performed. The field of view, matrix, number of excitations, phase encode steps, use of fat sup pression and fast sequences should be optimised for the spe

cific body part being imaged as well as the scanner utilised. It is beyond the scope of this document or appendix to pre scribe specific MRI pulse sequence parameters for all scan ners, body parts and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath hold scanning tech niques if possible.

Selection of target lesions: In general, the *largest* lesions rep resentative of involved organs (up to a maximum of two per organ and five total) are selected to follow as target lesions. However, in some cases, the largest lesions may not be easily measured and are not suitable for follow up because of their configuration. In these cases, identification of the largest *most reproducible* lesions is advised. Fig. 3 provides an illustrative example where the largest lesion is not the most reproducible and another lesion is better to select and follow:

Measurement of lesions

The longest diameter of selected lesions should be measured in the plane in which the images were acquired. For body CT, this is the axial plane. In the event isotropic reconstructions are performed, measurements can be made on these recon structed images; however, it should be cautioned that not all radiology sites are capable of producing isotropic recon structions. This could lead to the undesirable situation of measurements in the axial plane at one assessment point and in a different plane at a subsequent assessment. There are some tumours, for instance paraspinal lesions, which are better measured in the coronal or sagittal plane. It would be acceptable to measure these lesions in these planes if the reconstructions in those planes were isotropic or the images were acquired with MRI in those planes. Using the same plane of evaluation, the maximal diameter of each target lesion should always be measured at subsequent follow up time points even if this results in measuring the lesion at a differ ent slice level or in a different orientation or vector compared with the baseline study. Software tools that calculate the maximal diameter for a perimeter of a tumour may be em ployed and may even reduce variability.

The only exception to the longest diameter rule is lymph node measurement. Because malignant nodes are identified by the length of their short axis, this is the guide used to determine not only whether they are pathological but is also the dimension measured for adding into the sum of target le sions. Fig. 4 illustrates this point: the large arrow identifies a malignant node: the shorter perpendicular axis is $\geqslant$15 mm and will be recorded. Close by (small arrow) there is a normal node: note here the long axis is greater than 10 mm but the short axis is well below 10 mm. This node should be consid ered non pathological.

If a lesion disappears and reappears at a subsequent time point it should continue to be measured. However, the pa tient's response at the point in time when the lesion reap pears will depend upon the status of his/her other lesions. For example, if the patient's tumour had reached a CR status and the lesion reappeared, then the patient would be consid ered PD at the time of reappearance. In contrast, if the tumour status was a PR or SD and one lesion which had disappeared then reappears, its maximal diameter should be added to the sum of the remaining lesions for a calculated response: in other words, the reappearance of an apparently 'disappeared' single lesion amongst many which remain is not in itself en

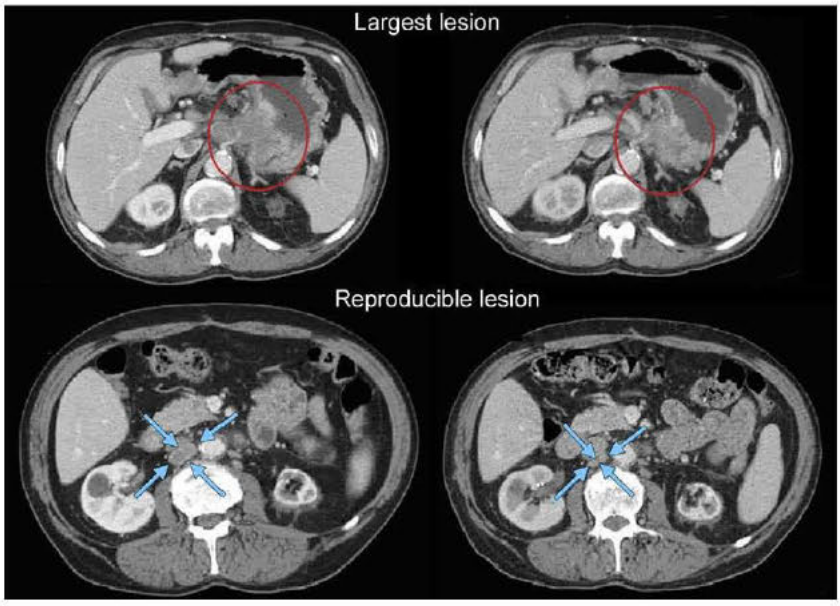

Fig. 3 – Largest lesion may not be most reproducible: most reproducible should be selected as target. In this example, the primary gastric lesion (circled at baseline and at follow-up in the top two images) may be able to be measured with thin section volumetric CT with the same degree of gastric distention at baseline and follow-up. However, this is potentially challenging to reproduce in a multicentre trial and if attempted should be done with careful imaging input and analysis. The most reproducible lesion is a lymph node (circled at baseline and at follow-up in the bottom two images).

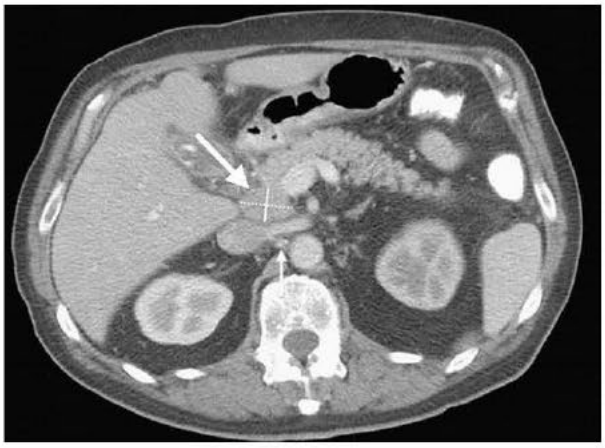

Fig. 4 – Lymph node assessment: large arrow illustrates a pathological node with the short axis shown as a solid line which should be measured and followed. Small arrow illustrates a non-pathological node which has a short axis <10 mm.

ough to qualify for PD: that requires the sum of all lesions to meet the PD criteria. The rationale for such a categorisation is based upon the realisation that most lesions do not actually 'disappear' but are not visualised because they are beyond the resolving power of the imaging modality employed.

The identification of the precise boundary definition of a lesion may be difficult especially when the lesion is embedded in an organ with a similar contrast such as the liver, pancreas, kidney, adrenal or spleen. Additionally, peritumoural oedema may surround a lesion and may be difficult to distinguish on certain modalities between this oedema and actual tumour. In fact, pathologically, the presence of tumour cells within the oedema region is variable. Therefore, it is most critical that the measurements be obtained in a reproducible manner from baseline and all subsequent follow up time points. This is also a strong reason to consistently utilise the same imaging modality.

When lesions 'fragment', the individual lesion diameters should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'merged lesion'.

Progression of non-target lesions

To achieve 'unequivocal progression' there must be an *overall* level of substantial worsening in non target disease that is of a magnitude that, even in the presence of SD or PR in target disease, the treating physician would feel it important to change therapy. Examples of unequivocal progression are shown in Figs. 5 and 6.

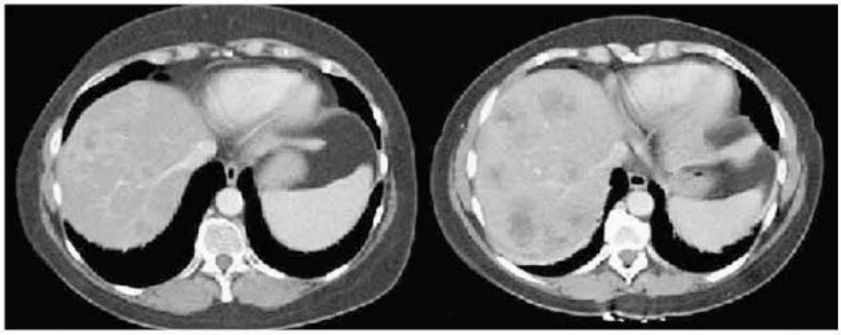

Fig. 5 – Example of unequivocal progression in non-target lesions in liver.

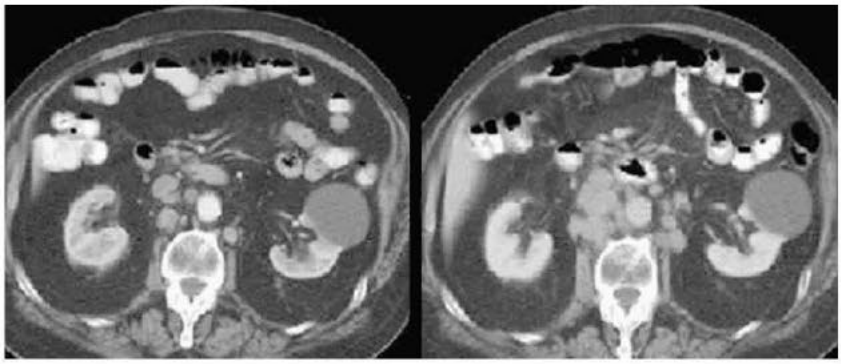

Fig. 6 – Example of unequivocal progression in non-target lesion (nodes).

Appendix III. Frequently asked questions

Question	Answer
What should be done if several unique lesions at baseline become confluent at a follow up evaluation?	Measure the longest diameter of the confluent mass and record to add into the sum of the longest diameters
How large does a new lesion have to be to count as progression? Does any small subcentimetre lesion qualify, or should the lesion be at least measurable?	New lesions do not need to meet 'measurability criteria' to be considered valid. If it is clear on previous images (with the same technique) that a lesion was absent then its definitive appearance implies progression. If there is any doubt (because of the techniques or conditions) then it is suggested that treatment continue until next scheduled assessment when, generally, all should be clear. Either it gets bigger and the date of progression is the date of the first suspicion, or it disappears and one may then consider it an artefact with the support of the radiologists
How should one lesion be measured if on subsequent exams it is split into two?	Measure the longest diameter of each lesion and add this into the sum
Does the definition of progression depend on the status of all target lesions or only one?	As per the RECIST 1.1 guideline, progression requires a 20% increase in the sum of diameters of all target lesions AND a minimum absolute increase of 5 mm in the sum
Are RECIST criteria accepted by regulatory agencies?	Many cooperative groups and members of pharma were involved in preparing RECIST 1.0 and have adopted them. The FDA was consulted in their development and supports their use, though they don't require it. The European and Canadian regulatory authorities also participated and the RECIST criteria are now integrated in the European note for guidance for the development of anticancer agents. Many pharmaceutical companies are also using them. RECIST 1.1 was similarly widely distributed before publication
What is the criterion for a measurable lesion if the CT slice thickness is >5 mm?	RECIST 1.1 recommends that CT scans have a maximum slice thickness of 5 mm and the minimum size for a measurable lesion is twice that: 10 mm (even if slice thickness is <5 mm). If scanners with slice thickness >5 mm are used, the minimum lesion size must have a longest diameter twice the actual slice thickness
What should we record when target lesions become so small they are below the 10 mm 'measurable' size?	Target lesion measurability is defined at baseline. Thereafter, actual measurements, even if <10 mm, should be recorded. If lesions become very small, some radiologists indicate they are 'too small to measure'. This guideline advises that when this occurs, if the lesion is actually still present, a default measurement of 5 mm should be applied. If in fact the radiologist believes the lesion has gone, a default measurement of 0 mm should be recorded
If a patient has several lesions which have decreased in size to meet PR criteria and one has actually disappeared, does that patient have PD if the 'disappeared' lesion reappears?	Unless the sum meets the PD criteria, the reappearance of a lesion in the setting of PR (or SD) is not PD. The lesion should simply be added into the sum. If the patients had had a CR, clearly reappearance of an absent lesion would qualify for PD
When measuring the longest diameter of target lesions in response to treatment, is the same axis that was used initially used subsequently, even if there is a shape change to the lesion that may have produced a new longest diameter?	The longest diameter of the lesion should always be measured even if the actual axis is different from the one used to measure the lesion initially (or at different time point during follow up) The only exception to this is lymph nodes: as per RECIST 1.1 the short axis should always be followed and as in the case of target lesions, the vector of the short axis may change on follow up
Target lesions have been selected at baseline and followed but then one of these target lesions then becomes non evaluable (i.e. different technique used) What is the effect this has on the other target lesions and the overall response?	What may be done in such cases is one of the following: (a) If the patient is still being treated, call the centre to be sure that future evaluations are done with the baseline technique so at least SOME courses are fully evaluable (b) If that is not possible, check if there IS a baseline exam by the same technique which was used to follow patients...in which case if you retrieve the baseline measures from that technique you retrieve the lesion evaluability (c) If neither (a) nor (b) is possible then it is a judgement call about whether you delete the lesion from all forms or consider the impact of the lesion overall is so important that its being non evaluable makes the overall response interpretation inevaluable without it. Such a decision should be discussed in a review panel It is NOT recommended that the lesion be included in baseline sums and then excluded from follow up sums since this biases in favour of a response

(continued on next page)

Appendix III – *continued*

Question	Answer
What if a single non target lesion cannot be reviewed, for whatever reason; does this negate the overall assessment?	Sometimes the major contribution of a single non target lesion may be in the setting of CR having otherwise been achieved: failure to examine one non target in that setting will leave you unable to claim CR. It is also possible that the non target lesion has undergone such substantial progression that it would override the target disease and render patient PD. However, this is very unlikely, especially if the rest of the measurable disease is stable or responding
A patient has a 32% decrease in sum cycle 2, a 28% decrease cycle 4 and a 33% decrease cycle 6. Does confirmation of PR have to take place in sequential scans or is a case like this confirmed PR?	It is not infrequent that tumour shrinkage hovers around the 30% mark. In this case, most would consider PR to have been confirmed looking at this overall case. Had there been two or three non PR observations between the two time point PR responses, the most conservative approach would be to consider this case SD
In the setting of a breast cancer neoadjuvant study, would mammography not be used to assess lesions? Is CT preferred in this setting?	Neither CT nor mammography are optimal in this setting. MRI is the preferred modality to follow breast lesions in a neoadjuvant setting
A patient has a lesion measurable by clinical exam and by CT scan. Which should be followed?	CT scan. Always follow by imaging if that option exists since it can be reviewed and verified
A lesion which was solid at baseline has become necrotic in the centre. How should this be measured?	The longest diameter of the entire lesion should be followed. Eventually, necrotic lesions which are responding to treatment decrease in size. In reporting the results of trials, you may wish to report on this phenomenon if it is seen frequently since some agents (e.g. angiogenesis inhibitors) may produce this effect
If I am going to use MRI to follow disease, what is minimum size for measurability?	MRI may be substituted for contrast enhanced CT for some sites, but not lung. The minimum size for measurability is the same as for CT (10 mm) as long as the scans are performed with slice thickness of 5 mm and no gap. In the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness. In the event there are inter slice gaps, this also needs to be considered in determining the size of measurable lesions at baseline
Can PET CT be used with RECIST?	At present, the low dose or attenuation correction CT portion of a combined PET CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if your site has documented that the CT performed as part of a PET CT is of the same diagnostic quality as a diagnostic CT (with IV and oral contrast) then the PET CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed

REFERENCES

1. Paesmans M, Sculier JP, Libert P, et al. Response to chemotherapy has predictive value for further survival of patients with advanced non small cell lung cancer: 10 years experience of the European Lung Cancer Working Party. *Eur J Cancer* 1997;**33**:2326 32.
2. Buyse M, Thirion P, Carlson RW, et al. Relation between tumor response to first line chemotherapy and survival in advanced colorectal cancer: a meta analysis. Meta analysis group in Cancer. *Lancet* 2000;**356**:373 8.
3. Goffin J, Baral S, Tu D, et al. Objective responses in patients with malignant melanoma or renal cell cancer in early clinical studies do not predict regulatory approval. *Clin Cancer Res* 2005;**15**:5928 34.
4. El Maraghi RH, Eisenhauer EA. Review of phase II trial designs used in studies of molecular targeted agents: outcomes and predictors of success in phase III. *J Clin Oncol* 2008;**10**:1346 54.
5. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;**47**:207 14.
6. Tonkin K, Tritchler D, Tannock I. Criteria of tumor response used in clinical trials of chemotherapy. *J Clin Oncol* 1985;**3**:870 5.
7. Baar J, Tannock I. Analyzing the same data in two ways: a demonstration model to illustrate the reporting and misreporting of clinical trials. *J Clin Oncol* 1989;**7**:969 78.
8. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors (RECIST Guidelines). *J Natl Cancer Inst* 2000;**92**:205 16.
9. Therasse P, Eisenhauer EA, Verweij J. RECIST revisited: a review of validation studies on tumour assessment. *Eur J Cancer* 2006;**42**:1031 9.
10. Bogaerts J, Ford R, Sargent D, et al. Individual patient data analysis to assess modifications to the RECIST criteria. *Eur J Cancer* 2009;**45**:248 60.
11. Moskowitz CS, Jia X, Schwartz LH, Gönen M. A simulation study to evaluate the impact of the number of lesions measured on response assessment. *Eur J Cancer* 2009;**45**:300 10.
12. Sargent D, Rubinstein L, Schwartz L, et al. Validation of novel imaging methodologies for use as cancer clinical trials end points. *Eur J Cancer* 2009;**45**:290 9.

13. Macdonald DR, Cascino TL, Schold Jr SC, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990;**8**:1277 80.
14. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol* 2007;**10**:579 86.
15. Schwartz LH, Bogaerts J, Ford R, et al. Evaluation of lymph nodes with RECIST 1.1. *Eur J Cancer* 2009;**45**:261 7.
16. Rustin GJ, Quinn M, Thigpen T, et al. Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). *J Natl Cancer Inst* 2004;**96**:487 8.
17. Bubley GJ, Carducci M, Dahut W, et al. Eligibility and response guidelines for phase II clinical trials in androgen independent prostate cancer: recommendations from the Prostate Specific Antigen Working Group. *J Clin Oncol* 1999;**17**:3461 7.
18. Scher H, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol* 2008;**26**:1148 59.
19. Vergote I, Rustin GJ, Eisenhauer EA, et al. Re: new guidelines to evaluate the response to treatment in solid tumors [ovarian cancer]. Gynecologic Cancer Intergroup. *J Natl Cancer Inst* 2000;**92**:1534 5.
20. Van Glabbeke M, Verweij J, Judson I, Nielsen OS. EORTC Soft Tissue and Bone Sarcoma Group: Progression free rate as the principal end point for phase II trials in soft tissue sarcomas. *Eur J Cancer* 2002;**38**:543 9.
21. Dancey JE, Dodd LE, Ford R, et al. Recommendations for the assessment of progression in randomised cancer treatment trials. *Eur J Cancer* 2009;**45**:281 9.
22. Ford R, Schwartz L, Dancey J, et al. Lessons learned from independent central review. *Eur J Cancer* 2009;**45**: 268 74.
23. Catalano C, Francone M, Ascarelli A, Mangia M, Iacucci I, Passariello R. Optimizing radiation dose and image quality. *Eur Radiol* 2007;**17**(Suppl 6):F26 32.
24. Low RN. Abdominal MRI advances in the detection of liver tumours and characterization. *Lancet Oncol* 2007;**8**(6):525 35.
25. Barrett T, Choyke PL, Kobayashi H. Imaging of the lymphatic system: new horizons. *Contrast Media Mol Imaging* 2006;**1**(6):230 45.
26. Shankar LK, Hoffman JM, Bacharach S, et al. National Cancer Institute. Consensus recommendations for the use of 18F FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. *J Nucl Med* 2006;**47**(6):1059 66.

Appendix 6. Response Assessment Using RANO Criteria

Response Assessment Using RANO Criteria

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SPECIAL ARTICLE

Updated Response Assessment Criteria for High-Grade Gliomas: Response Assessment in Neuro-Oncology Working Group

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ABSTRACT

Currently, the most widely used criteria for assessing response to therapy in high-grade gliomas are based on two-dimensional tumor measurements on computed tomography (CT) or magnetic resonance imaging (MRI), in conjunction with clinical assessment and corticosteroid dose (the Macdonald Criteria). It is increasingly apparent that there are significant limitations to these criteria, which only address the contrast-enhancing component of the tumor. For example, chemoradiotherapy for newly diagnosed glioblastomas results in transient increase in tumor enhancement (pseudoprogression) in 20% to 30% of patients, which is difficult to differentiate from true tumor progression. Antiangiogenic agents produce high radiographic response rates, as defined by a rapid decrease in contrast enhancement on CT/MRI that occurs within days of initiation of treatment and that is partly a result of reduced vascular permeability to contrast agents rather than a true antitumor effect. In addition, a subset of patients treated with antiangiogenic agents develop tumor recurrence characterized by an increase in the nonenhancing component depicted on T2-weighted/fluid-attenuated inversion recovery sequences. The recognition that contrast enhancement is nonspecific and may not always be a true surrogate of tumor response and the need to account for the nonenhancing component of the tumor mandate that new criteria be developed and validated to permit accurate assessment of the efficacy of novel therapies. The Response Assessment in Neuro-Oncology Working Group is an international effort to develop new standardized response criteria for clinical trials in brain tumors. In this proposal, we present the recommendations for updated response criteria for high-grade gliomas.

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INTRODUCTION

Gliomas are the most common form of malignant primary brain tumors in adults, with an annual incidence of approximately four to five per 100,000 people.[1,2] The evaluation of treatment in high-grade gliomas currently relies either on the duration of patient survival or, more commonly in patients with recurrent disease, the radiographic response rate or progression-free survival (PFS).[3,4] In 1990, Macdonald et al[5] published criteria for response assessment in high-grade gliomas (Table 1). These criteria provided an objective radiologic assessment of tumor response and were based primarily on contrast-enhanced computed tomography (CT) and the two-dimensional WHO oncology response criteria using enhancing tumor area (the product of the maximal cross-sectional enhancing diameters) as the primary tumor measure.[6,7] These criteria also considered the use of corticosteroids and changes in the neurologic status of the patient. The Macdonald Criteria enabled response rates to be compared between clinical trials and have been widely used in high-grade glioma studies since their introduction.

Although the Macdonald Criteria were developed primarily for CT scans, they have been extrapolated to magnetic resonance imaging (MRI), which is now the standard neuroimaging modality used to assess treatment response in high-grade gliomas. Like CT scans, areas of the tumor with abnormal vascular architecture and disrupted integrity of the blood-brain barrier are depicted as the contrast-enhancing component on MRI.[8]

In systemic cancers, one-dimensional tumor measurements have become the standard criteria to determine response. The Response Evaluation Criteria in Solid Tumors (RECIST) first introduced the use of one-dimensional measurements in 2000[9] and were recently revised (RECIST version 1.1).[10] Several studies have compared the RECIST criteria with

Table 1. Current Response Criteria for Malignant Gliomas (Macdonald Criteria)[5]

Response	Criteria
Complete response	Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; no corticosteroids; and stable or improved clinically
Partial response	Requires all of the following: ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks; no new lesions; stable or reduced corticosteroid dose; and stable or improved clinically
Stable disease	Requires all of the following: does not qualify for complete response, partial response, or progression; and stable clinically
Progression	Defined by any of the following: ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions; any new lesion; or clinical deterioration

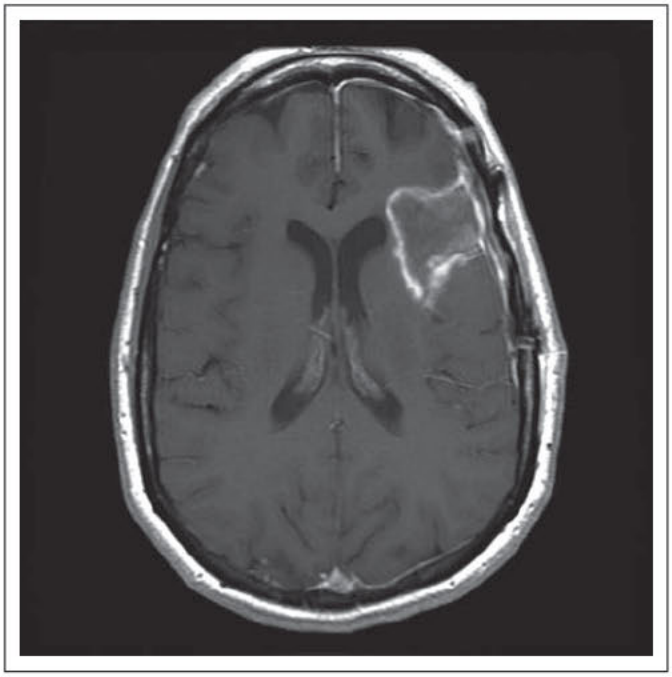

Fig 1. A 38-year-old patient with left frontal glioblastoma showing irregular enhancement in wall of the cavity that is difficult to measure. Although the entire cavity is often measured, it would be preferable if only the enhancing nodule in the posterior wall of the cavity were measured. If it is smaller than 10 mm in bidirectional diameters, the lesion would be considered nonmeasurable.

two-dimensional measurements, three-dimensional measurements, and volumetric measurements in high-grade gliomas.[11-13] These studies suggest that there is good concordance among the different methods in determining response in adult patients with both newly diagnosed and recurrent high-grade gliomas,[12,13] as well as in pediatric brain tumors.[11] However, an exception is seen with three-dimensional measurements, which seem to be inferior to one- and two-dimensional and volumetric measurements.[12,14] Nonetheless, studies prospectively validating the RECIST criteria in gliomas have not been performed. Currently, the Macdonald Criteria using two-dimensional measurement remain the most widely used method for evaluating tumor response in clinical trials of high-grade gliomas, partly because they enable the results of ongoing studies to be easily compared with historical data.

LIMITATIONS OF THE MACDONALD CRITERIA

From their inception, it was apparent that the Macdonald Criteria had a number of important limitations. These limitations, which have recently been reviewed in detail,[15-17] include the difficulty of measuring irregularly shaped tumors, interobserver variability, the lack of assessment of the nonenhancing component of the tumor, lack of guidance for the assessment of multifocal tumors, and the difficulty in measuring enhancing lesions in the wall of cystic or surgical cavities because the cyst/cavity itself may be included in the tumor measurement (Fig 1). In the Macdonald Criteria, a significant increase (at least 25%) in the contrast-enhancing lesion is used as a reliable surrogate marker for tumor progression, and its presence mandates a change in therapy. However, contrast enhancement is nonspecific and primarily reflects the passage of contrast material across a disrupted blood-tumor barrier. Enhancement can be influenced by changes in corticosteroid doses, antiangiogenic agents (discussed later), and changes in radiologic techniques.[18,19] Increased enhancement can also be induced by a variety of nontumoral processes such as treatment-related inflammation, seizure activity, postsurgical changes, ischemia, subacute radiation effects, and radiation necrosis.[20-23] As a result, there are significant limitations in equating changes in enhancing area with changes in tumor size or tumor growth. The limitations of the Macdonald Criteria have become even more apparent with the increased incidence of pseudoprogression in patients receiving radiotherapy with temozolomide and the recent introduction of antiangiogenic therapies that affect the permeability of tumor vasculature. This has led to the current effort to revise the response criteria for high-grade gliomas.[17] The major issues are discussed in the following sections.

Pseudoprogression and Radiation Effects

Standard therapy for glioblastoma involves maximal safe tumor resection followed by radiotherapy with concurrent and adjuvant temozolomide.[24,25] Twenty to 30% of patients undergoing their first postradiation MRI show increased contrast enhancement that eventually subsides without any change in therapy (Fig 2). This phenomenon, termed pseudoprogression, likely results from transiently increased permeability of the tumor vasculature from irradiation, which may be enhanced by temozolomide, and complicates the determination of tumor progression immediately after completion of radiotherapy.[26-30] Pseudoprogression may be accompanied by progressive clinical signs and symptoms and seems to be more frequent in patients with a methylated *MGMT* gene promoter.[30] This treatment-related effect has implications for patient management and may result in premature discontinuation of effective adjuvant therapy. This limits the validity of a PFS end point unless tissue-based confirmation of tumor progression is obtained. It also has significant implications for selecting appropriate patients for participation in clinical trials for recurrent gliomas. Failure to exclude patients with pseudoprogression from these studies will result in a falsely high response rate and PFS

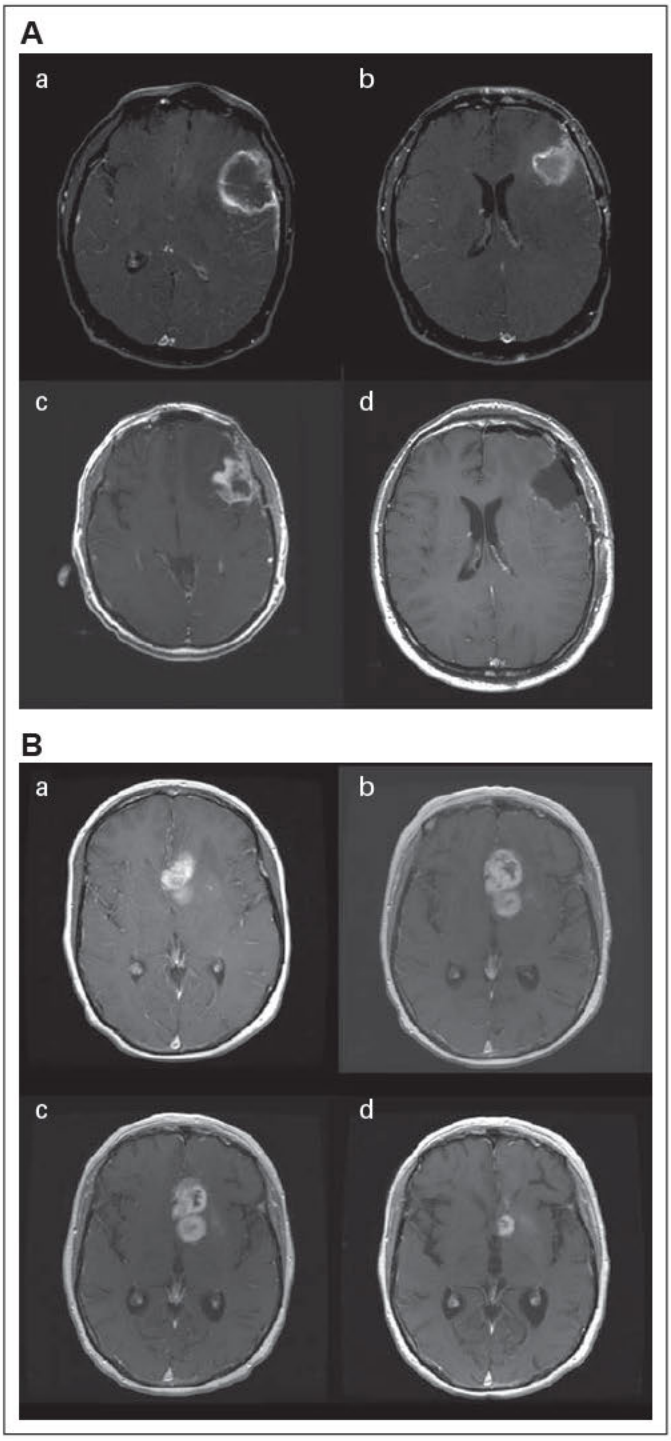

Fig 2. (A) Pseudoprogression after chemoradiotherapy: axial T1-contrast enhanced magnetic resonance imaging (MRI) a) before surgery; b) after surgery; c) after radiotherapy and concomitant temozolomide showing increased enhancement; d) re-operation showing only necrotic tissue and no tumor. (B) Pseudoprogression after chemoradiotherapy: axial T1-contrast enhanced MRI showing deep left frontal glioblastoma a) 2 days after stereotactic biopsy; b) 4 weeks after radiotherapy and concomitant temozolomide showing increased enhancement, raising the possibility of progression; c) after 4 additional weeks of treatment with adjuvant temozolomide showing stable disease; d) after 8 cycles of adjuvant temozolomide showing significant reduction in tumor size.

and the possibility that an agent will be incorrectly considered to be active. To address this issue, the proposed new response criteria suggest that within the first 12 weeks of completion of radiotherapy, when pseudoprogression is most prevalent, progression can only be determined if the majority of the new enhancement is outside of the radiation field (for example, beyond the high-dose region or 80% isodose line) or if there is pathologic confirmation of progressive disease (Table 2). It is recognized that the proposed histologic criteria have important limitations, but they provide guidance on the type of findings that are suggestive of progressive disease. For patients in whom pseudoprogression cannot be differentiated from true tumor progression, enrollment onto trials for recurrent gliomas should not be permitted. Patients who remain clinically stable and/or are suspected to have pseudoprogression based on metabolic or vascular imaging should continue with their current therapy.

Enhancement As a Result of Surgery and Other Therapies

Increased enhancement often develops in the wall of the surgical cavity 48 to 72 hours after surgery.[20,31-33] To avoid interpretation of

Table 2. Criteria for Determining First Progression Depending on Time From Initial Chemoradiotherapy

First Progression	Definition
Progressive disease < 12 weeks after completion of chemoradiotherapy	Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (eg, solid tumor areas [ie, > 70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note: Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of progressive disease in the first 12 weeks after completion of concurrent chemoradiotherapy.
Progressive disease ≥ 12 weeks after chemoradiotherapy completion	1. New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids. 2. Increase by ≥ 25% in the sum of the products of perpendicular diameters between the first postradiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids. 3. Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence. 4. For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR nonenhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (eg, effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).

Abbreviation: FLAIR, fluid-attenuated inversion recovery.

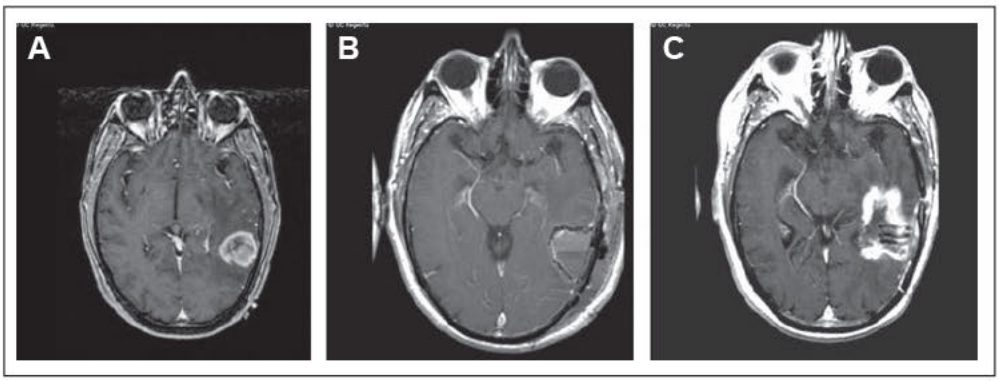

Fig 3. Pseudoprogression after brachytherapy. (A) Axial T1 contrast-enhanced magnetic resonance imaging (MRI) showing enhancing tumor before surgery. (B) Immediate postoperative magnetic resonance imaging (MRI) showing acute surgical changes and placement of iodine-125 brachytherapy seeds. (C) MRI performed 18 months later showing increased enhancement. Reoperation showed no tumor.

postoperative changes as residual enhancing disease, a baseline MRI scan should ideally be obtained within 24 to 48 hours after surgery and no later than 72 hours after surgery. The inclusion of diffusion-weighted imaging in the immediate postoperative MRI scan can be helpful in determining whether new enhancement developing in the subsequent weeks or months is caused by sequelae of ischemia or by tumor recurrence.[16,22] In addition, a transient increase in enhancement that can be difficult to distinguish from recurrent disease can also occur after locally administered therapies. These include chemotherapy wafers, immunotoxins delivered by convection-enhanced delivery, regionally administered gene and viral therapies, immunotherapies, and focal irradiation with brachytherapy and stereotactic radiosurgery (Fig 3).[17,34-38] Imaging modalities such as perfusion imaging, magnetic resonance spectroscopy, and positron emission tomography scans may sometimes be helpful in differentiating treatment effects from recurrent tumor.[39-42] However, no imaging modality currently has sufficient specificity to conclusively differentiate recurrent tumor from treatment effects, and surgical sampling may occasionally be needed to obtain a definitive diagnosis.

Pseudoresponses After Treatment With Antiangiogenic Therapies

Antiangiogenic agents, especially those targeting vascular endothelial growth factor (VEGF), such as bevacizumab, and the VEGF receptor, such as cediranib, can produce marked decrease in contrast enhancement as early as 1 to 2 days after initiation of therapy and commonly result in high radiologic response rates of 25% to 60%.[43-46] These apparent responses to antiangiogenic therapy may be partly a result of normalization of abnormally permeable tumor vessels and not always necessarily indicative of a true antiglioma effect (Fig 4). As a result, radiologic responses in studies with antiangiogenic agents should be interpreted with caution. There is a disappointing disparity between the unprecedented high response rates these agents produce in recurrent glioblastoma and the modest survival benefits, if any, that have been reported.[47] Although the duration of response or stability (PFS) or overall survival may be a more accurate indicator of a true anti-glioma effect, there is emerging data suggesting that the degree of initial response may also correlate with survival.[48] As with the Macdonald Criteria, the proposed criteria suggest that radiologic responses should persist for at least 4 weeks before they are considered as true responses.

Failure to Measure Nonenhancing Tumor

High-grade gliomas are infiltrative in nature, and their presence does not always result in disruption of the blood-brain barrier. In fact, determination of the extent of this nonenhancing component of the tumor, usually depicted on the MRI T2-weighted and fluid-attenuated inversion recovery (FLAIR) image sequences, can be difficult because peritumoral edema and delayed radiation white matter changes have similar radiographic appearances. Because the Macdonald Criteria do not account for the nonenhancing component of the tumor, this is especially problematic for low-grade gliomas (WHO grade 2) and anaplastic gliomas (WHO grade 3), where a significant portion of the tumor is typically nonenhancing.

As experience with antiangiogenic therapies has grown, especially with agents targeting VEGF and VEGF receptor, it has become apparent that a subset of patients who initially experience reduction in tumor contrast enhancement subsequently develop progressive increase in nonenhancing T2 or FLAIR signals suggestive of infiltrative tumor (Fig 5).[49-51] Increasing evidence suggests that anti-VEGF therapy may increase the tendency of tumor cells to co-opt existing blood vessels, resulting in an invasive nonenhancing phenotype.[52-54] Unlike the Macdonald Criteria, which do not take into account progressive nonenhancing disease, the new response assessment will consider enlarging areas of nonenhancing tumor as evidence of tumor progression (Tables 3 and 4). However, precise quantification of the increase in T2/FLAIR signal can be difficult and must be differentiated from other causes of increased T2 or FLAIR signal, such radiation effects, decreased corticosteroid dosing, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects, before making a determination of progressive disease. Changes in T2/FLAIR signal that suggest infiltrating tumor include mass effect (as determined by sulcal effacement, ventricular compression, and thickening of the corpus callosum), infiltration of the cortical ribbon, and location outside of the radiation field. Although it would be preferable to have an objective measure of progressive nonenhancing recurrent disease similar to contrast-enhancing disease, the Response Assessment in Neuro-Oncology (RANO) Working Group felt that this was not possible at present given the limitations of current technology.

The initiation of these changes can be subtle, and convincing non–contrast-enhancing growth may require one or two confirmatory scans. If nonenhancing progression is determined after retrospective review of images, the scan at which these changes were first detected should serve as the progression scan.

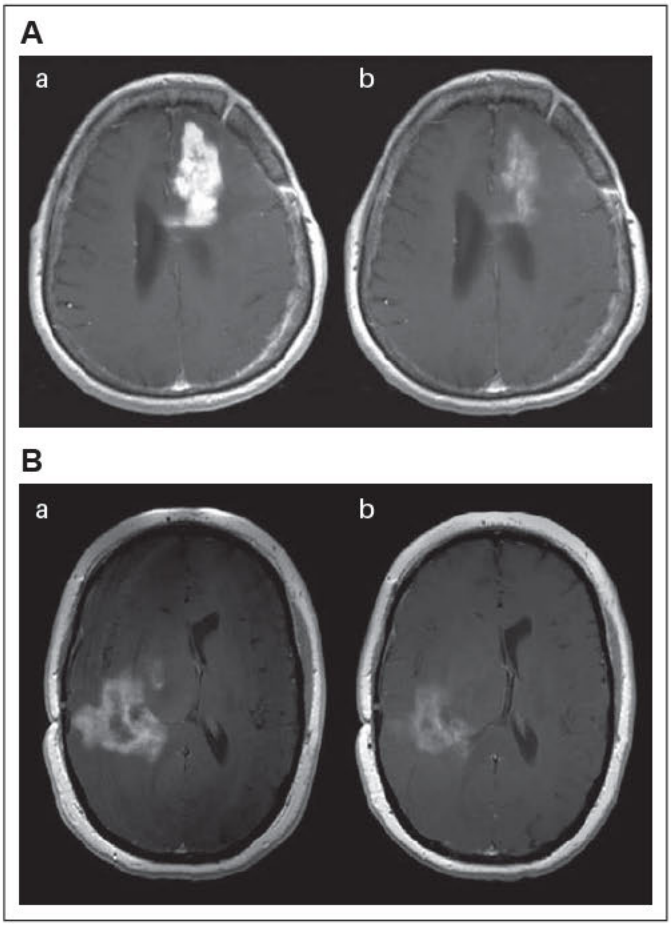

Fig 4. (A) Pseudoresponse. Axial T1-weighted contrast enhanced MRI of left frontal recurrent glioblastoma a) before and b) one day after therapy with cediranib (pan-VEGFR inhibitor) showing significant reduction in contrast enhancement. The reduction in contrast enhancement within 1 day of therapy is more likely to be caused by reduced vascular permeability to contrast than to a true antitumor effect. (Slide courtesy of A. Gregory Sorensen, Massachusetts General Hospital; Adapted with permission from Batchelor et al. Cancer Cell 11:83-95, 2007[43]). (B) Pseudoresponse. Axial T1-weighted contrast enhanced MRI of right parietal glioblastoma a) before and b) 1 day after therapy with XL184 (vascular endothelial growth factor receptor [VEGFR] and MET inhibitor) showing significant reduction in contrast enhancement. (Slide courtesy of A. Gregory Sorensen, Massachusetts General Hospital).

Progressive nonenhancing tumor is often associated with neurologic deterioration, and consequently, the clinical status of the patients may help in determining progressive disease. Given the lack of validated measures of neurologic function, a precise definition of neurologic deterioration is not included in the proposed response criteria. However, it is recommended that a decline in the Karnofsky performance score (KPS), Eastern Cooperative Oncology Group performance status, or WHO performance score be considered in determining clinical deterioration. The specific details are discussed later in the section defining progression.

PROCESS OF DEVELOPMENT OF THE UPDATED RESPONSE CRITERIA IN HIGH-GRADE GLIOMAS

Because of the limitations of the Macdonald Criteria, there has been an international effort in neuro-oncology to improve imaging response assessments for high-grade glioma and to enhance the interpretation of clinical trials involving novel agents that affect the blood-brain barrier such as antiangiogenic therapies. The RANO Working Group consists of neuro-oncologists, neurosurgeons, radiation oncologists, neuroradiologists, neuropsychologists, and experts in quality-of-life measures, in collaboration with government and industry. The RANO Working Group includes members with leadership roles in the major neuro-oncology organizations and brain tumor cooperative groups in both the United States and Europe. Recognizing the challenges in other neuro-oncologic clinical scenarios, imaging response recommendations are also being generated for low-grade glioma and the evaluation of surgically based therapies and will be reported separately.

In the following section, we outline a proposal for updated response criteria in high-grade gliomas from the RANO Working Group. It must be emphasized that this represents a work in progress. In coming years, as new volumetric and physiologic imaging techniques (eg, perfusion, permeability, and diffusion imaging; magnetic resonance spectroscopy; and metabolic imaging)[55,56] and other end points such as neuropsychological testing and quality-of-life measures are developed and validated in neuro-oncology, the RANO Working Group anticipates incorporating these parameters into the response criteria.

STANDARDIZATION OF IMAGING DEFINITIONS

Specific lesions must be evaluated serially, and comparative analysis of changes in the area of contrast enhancement, as well as the nonenhancing component, should be performed. As with the Macdonald Criteria, the product of the maximal cross-sectional enhancing diameters will be used to determine the size of the contrast-enhancing lesions.

Measureable and Nonmeasurable Disease for Contrast-Enhancing Lesions

Measurable disease is defined as bidimensionally contrast-enhancing lesions with clearly defined margins by CT or MRI scan, with two perpendicular diameters of at least 10 mm, visible on two or more axial slices that are preferably, at most, 5 mm apart with 0-mm skip. As with RECIST version 1.1, in the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness.[10] In the event there are interslice gaps, this also needs to be considered in determining the size of measurable lesions at baseline. Measurement of tumor around a cyst or surgical cavity represents a particularly difficult challenge. In general, such lesions should be considered nonmeasurable unless there is a nodular component measuring $\geq$ 10 mm in diameter. The cystic or surgical cavity should not be measured in determining response.

Nonmeasurable disease is defined as either unidimensionally measurable lesions, masses with margins not clearly defined, or lesions with maximal perpendicular diameters less than 10 mm.

Patients without measurable disease, such as those who undergo a gross total resection, cannot respond and can only achieve stable disease as their best radiographic outcome. Therefore, if response rate is the primary end point of the study, patients with measurable disease are required for study eligibility. If duration of

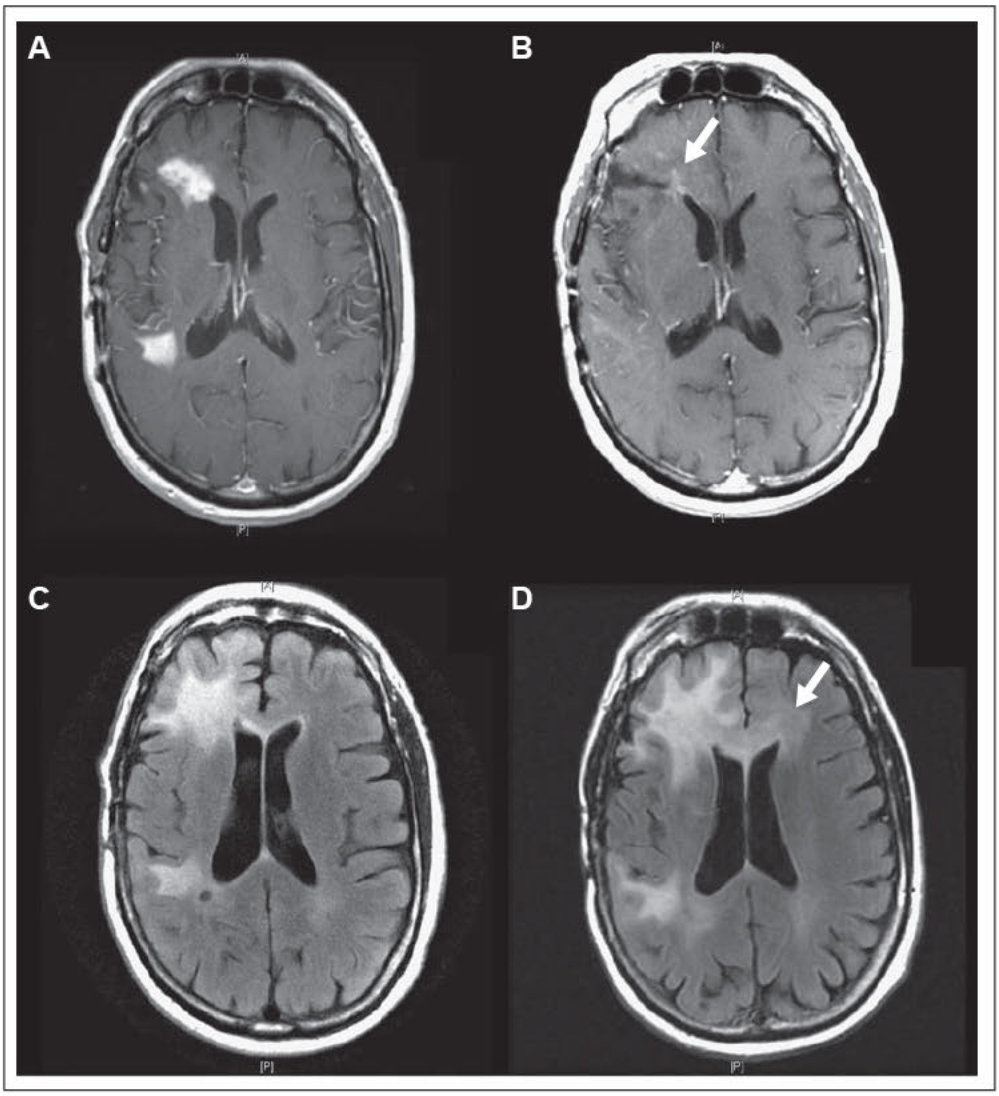

Fig 5. A 54-year-old patient with recurrent glioblastoma showing nonenhancing progression after bevacizumab therapy. Axial contrast-enhanced, T1-weighted images show (A) scan at recurrence showing multifocal right frontal glioblastoma; (B) decreased enhancement after 7 months of therapy that qualifies by Macdonald Criteria as partial response; (C) axial fluid-attenuated inversion recovery image at baseline and (D) after 7 months of therapy showing nonenhancing tumor progressing through corpus callosum to the left frontal lobe.

tumor control or survival is the primary end point, then patients with both measurable and nonmeasurable disease would be eligible for assessment because the determination of disease progression would be the primary interest.

Number of Lesions

If there are multiple contrast-enhancing lesions, a minimum of the two largest lesions should be measured, and the sum of the products of the perpendicular diameters of these lesions should be determined, similar to the criteria proposed for systemic tumors in RECIST version 1.1.[10] However, given the heterogeneity of high-grade gliomas and the difficulty in measuring some lesions, a maximum of five of the largest lesions may be measured. In general, the largest enlarging lesion(s) should be selected. However, emphasis should also be placed on lesions that allow reproducible repeated measurements. Occasionally, the largest lesions may not lend themselves to reproducible measurements, and the next largest lesions that can be measured reproducibly should be selected.

For patients with recurrent disease who have multiple lesions of which only one or two are increasing in size, the enlarging lesions should be considered the target lesions for evaluation of response. The other lesions will be considered nontarget lesions and should also be recorded. Rarely, unequivocal progression of a nontarget lesion requiring discontinuation of therapy or development of a new contrast-enhancing lesion may occur, even in the setting of stable disease or partial response in the target lesions. These changes would qualify as progression.

CRITERIA FOR DETERMINING FIRST PROGRESSION DEPENDING ON TIME FROM INITIAL CHEMORADIOTHERAPY

As mentioned earlier, 20% to 30% of patients develop pseudoprogression after chemoradiotherapy, especially within the first 3 months after completion of radiotherapy.[27] Given the difficulty of differentiating pseudoprogression from true progression in the first 12 weeks after irradiation, we propose excluding these patients from clinical trials for recurrent disease unless the progression is clearly outside the radiation field (eg, beyond the high-dose region or 80% isodose line)

Table 3. Criteria for Response Assessment Incorporating MRI and Clinical Factors

Response	Criteria
Complete response	Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions; patients must be off corticosteroids (or on physiologic replacement doses only); and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks; no progression of nonmeasurable disease; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan; and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: does not qualify for complete response, partial response, or progression; stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*; significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (eg, radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects); any new lesion; clear clinical deterioration not attributable to other causes apart from the tumor (eg, seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose; failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.

NOTE. All measurable and nonmeasurable lesions must be assessed using the same techniques as at baseline.
Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
*Stable doses of corticosteroids include patients not on corticosteroids.

or there is pathologic confirmation of disease progression. Table 2 lists these recommendations.

CRITERIA FOR ENTRY ONTO CLINICAL TRIALS FOR RECURRENT HIGH-GRADE GLIOMA

Currently, patients with any worsening of their imaging studies are eligible for entry onto clinical trials for recurrent gliomas, even if the change is minimal. We propose that patients should be required to have a 25% increase in the sum of the products of perpendicular diameters of the contrast-enhancing lesions, while on stable or increasing doses of corticosteroids, before they are considered to have progressive disease and are entered onto clinical trials for recurrent/progressive disease. Patients with new contrast-enhancing nonmeasurable disease may be considered for clinical trials in which PFS is the primary end point. Clinical deterioration or increase in corticosteroid dosing alone would not be sufficient to indicate progressive disease for entry onto clinical studies.

A particularly difficult problem involves patients receiving first-line antiangiogenic agents who develop predominantly nonenhancing disease at progression. This can be difficult to differentiate from treatment effects. If it seems clear that the nonenhancing changes represent tumor progression, these patients would also be eligible for enrollment onto clinical trials for recurrent disease, although their tumor will be considered nonmeasurable. As noted previously, although it would be preferable to have a more objective measure of progressive nonenhancing recurrent disease similar to contrast-enhancing disease, the RANO Working Group felt that this was not possible at present given the limitations of current technology.

DEFINITION OF RADIOGRAPHIC RESPONSE

Radiographic response should be determined in comparison to the tumor measurement obtained at pretreatment baseline for determination of response, and the smallest tumor measurement at either pretreatment baseline or after initiation of therapy should be used for determination of progression. Table 3 lists the criteria for radiographic changes after therapy. In the event that the radiographic changes are equivocal and it is unclear whether the patient is stable or has developed progressive disease, it is permissible to continue treatment and observe the patient closely, for example at 4-week intervals. If subsequent imaging studies demonstrate that progression has occurred, the date of progression should be the date of the scan at which this issue was first raised. The determination of radiographic response after treatment with agents, such as antiangiogenic therapies, that affect vascular permeability is particularly difficult. In these patients, consideration should be given to performing a second scan at 4 weeks to confirm the presence of response or stable disease.

All measurable and nonmeasurable lesions should be assessed using the same techniques as at baseline. Ideally, patients should be imaged on the same MRI scanner, or at least with the same magnet strength, for the duration of the study to reduce difficulties in interpreting changes.

Complete Response

Complete response requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions; and patient must be off corticosteroids or on physiologic replacement doses only, and stable or improved clinically. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease.

Partial Response

Partial response requires all of the following: ≥ 50% decrease, compared with baseline, in the sum of products of perpendicular

Table 4. Summary of the Proposed RANO Response Criteria

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.
*Progression occurs when this criterion is present.
†Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

diameters of all measurable enhancing lesions sustained for at least 4 weeks; no progression of nonmeasurable disease; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; and patient must be on a corticosteroid dose not greater than the dose at time of baseline scan and is stable or improved clinically. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease.

Stable Disease

Stable disease occurs if the patient does not qualify for complete response, partial response, or progression (see next section) and requires the following: stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan and clinically stable status. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

Progression

Progression is defined by any of the following: ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions (compared with baseline if no decrease) on stable or increasing doses of corticosteroids; a significant increase in T2/FLAIR nonenhancing lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy, not due to comorbid events; the appearance of any new lesions; clear progression of nonmeasurable lesions; or definite clinical deterioration not attributable to other causes apart from the tumor, or to decrease in corticosteroid dose. Failure to return for evaluation as a result of death or deteriorating condition should also be considered as progression.

Increase in corticosteroid dose alone, in the absence of clinical deterioration related to tumor, will not be used as a determinant of progression. Patients with stable imaging studies whose corticosteroid dose was increased for reasons other than clinical deterioration related to tumor do not qualify for stable disease or progression. They should be observed closely. If their corticosteroid dose can be reduced back to baseline, they will be considered as having stable disease; if further clinical deterioration related to tumor becomes apparent, they will be considered to have progression. The date of progression should be the first time point at which corticosteroid increase was necessary.

The definition of clinical deterioration is left to the discretion of the treating physician, but it is recommended that a decline in the KPS from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration unless attributable to comorbid events or changes in corticosteroid dose. Similarly, a decline in the Eastern Cooperative Oncology Group and WHO performance scores from 0 or 1 to 2 or 2 to 3 would be considered neurologic deterioration.

Patients with nonmeasurable enhancing disease whose lesions have significantly increased in size and become measurable (minimal bidirectional diameter of ≥ 10 mm and visible on at least two axial slices that are preferably, at most, 5 mm apart with 0-mm skip) will also be considered to have experienced progression. The transition from a nonmeasurable lesion to a measurable lesion resulting in progression can theoretically occur with relatively small increases in tumor size (eg, a 9 × 9 mm lesion [nonmeasurable] increasing to a 10 × 11 mm lesion [measurable]). Ideally, the change should be significant (> 5 mm increase in maximal diameter or ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions). In general, if there is doubt about whether the lesion has progressed, continued treatment and close follow-up evaluation will help clarify whether there is true progression.

If there is uncertainty regarding whether there is progression, the patient may continue on treatment and remain under close observation (eg, evaluated at 4-week intervals). If subsequent evaluations suggest that the patient is in fact experiencing progression, then the date of progression should be the time point at which this issue was first raised.

MULTIFOCAL TUMORS

For multifocal lesions, progressive disease is defined as ≥ 25% increase in the sum of products of perpendicular diameters of all measurable lesions compared with the smallest tumor measurements after initiation of therapy (Table 3). The appearance of a new lesion or unequivocal progression of nontarget lesions will also be considered progression. Partial response is defined as ≥ 50% decrease, compared with baseline, in the sum of products of perpendicular diameters of all measurable lesions sustained for at least 4 weeks with stable or decreasing corticosteroid doses.

ROLE OF VOLUMETRIC AND ADVANCED MRI ASSESSMENT

Given the limitations of two-dimensional tumor measurements, there is significant interest in volumetric anatomic assessment. The use of volumetric assessment would allow more accurate determination of the contrast-enhancing and nonenhancing volumes and overcome the limitations of two-dimensional measurements of lesions surrounding a surgical cavity.[14-16] However, the RANO Working Group and colleagues in neuroradiology do not believe that there is sufficient standardization and availability to recommend adoption of volumetric assessment of tumor volume at present. Nonetheless, this is an important area of research. Eventually, as volumetric imaging becomes more standardized and widely available and as data validating this approach emerge, it may be possible to incorporate volumetric measurements in the response assessment of high-grade gliomas.

Emerging data also suggest that advanced MRI techniques such as perfusion imaging (dynamic susceptibility MRI), permeability imaging (dynamic contrast-enhanced MRI), diffusion imaging, magnetic resonance spectroscopy, and [^{18}F]-fluorothymidine and amino acid positron emission tomography may predict tumor response or allow the differentiation of nonenhancing tumor from other causes of increased FLAIR signal. These techniques will require rigorous clinical validation studies before they can be incorporated into response criteria used in clinical trials in high-grade gliomas.

OTHER METHODS OF DETERMINING EFFICACY

Growing data suggest that other end points such as neurocognitive function, quality of life, and corticosteroid use may be used to measure clinical benefit. At present, these end points are not sufficiently validated to be incorporated into the current response criteria but could be added in the future as further data emerge.

CONCLUSION

We propose updated response assessments for the evaluation of therapies in high-grade gliomas incorporating MRI characteristics to address the recognized and accepted limitations of the current Macdonald Criteria. These recommendations were generated as part of an international neuro-oncology effort with consensus building and are an attempt to develop standardized assessment criteria. Implementation into future clinical trials will be critical so we can validate the criteria as a surrogate to end points such as survival and, ultimately, improve the accuracy and efficiency of the early evaluation of novel therapies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Central Brain Tumor Registry of the United States: Statistical report: Primary brain tumors in the United States, 2000-2004. http://www.cbtrus.org/reports/2007-2008/2007report.pdf
2. Wen PY, Kesari S: Malignant gliomas in adults. N Engl J Med 359:492-507, 2008
3. Wong ET, Hess KR, Gleason MJ, et al: Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. J Clin Oncol 17:2572-2578, 1999
4. Lamborn KR, Yung WK, Chang SM, et al: Progression-free survival: An important end point in evaluating therapy for recurrent high-grade gliomas. Neuro Oncol 10:162-170, 2008
5. Macdonald D, Cascino T, Schold SJ, et al: Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 8:1277-1280, 1990
6. WHO: WHO Handbook for Reporting Results of Cancer Treatment. World Health Organization Offset Publication No. 48. Geneva, Switzerland, WHO, 1979
7. Miller A, Hoogstraten B, Staquet M, et al: Reporting results of cancer treatment. Cancer 47: 207-214, 1981
8. Rees J: Advances in magnetic resonance imaging of brain tumours. Curr Opin Neurol 16:643-650, 2003
9. Therasse P, Arbuck S, Eisenhauer E, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for

Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000

10. Eisenhauer EA, Therasse P, Bogaerts J, et al: New Response Evaluation Criteria in Solid Tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009

11. Warren KE, Patronas N, Aikin AA, et al: Comparison of one-, two-, and three-dimensional measurements of childhood brain tumors. J Natl Cancer Inst 93:1401-1405, 2001

12. Shah GD, Kesari S, Xu R, et al: Comparison of linear and volumetric criteria in assessing tumor response in adult high-grade gliomas. Neuro Oncol 8:38-46, 2006

13. Galanis E, Buckner JC, Maurer MJ, et al: Validation of neuroradiologic response assessment in gliomas: Measurement by RECIST, two-dimensional, computer-assisted tumor area, and computer-assisted tumor volume methods. Neuro Oncol 8:156-165, 2006

14. Sorensen A, Patel S, Harmath C, et al: Comparison of diameter and perimeter methods for tumor volume calculation. J Clin Oncol 19:551-557, 2001

15. Sorensen AG, Batchelor TT, Wen PY, et al: Response criteria for glioma. Nat Clin Pract Oncol 5:634-644, 2008

16. Henson JW, Ulmer S, Harris GJ: Brain tumor imaging in clinical trials. Am J Neuroradiol 29:419-424, 2008

17. van den Bent MJ, Vogelbaum MA, Wen PY, et al: End point assessment in gliomas: Novel treatments limit usefulness of classical Macdonald's Criteria. J Clin Oncol 27:2905-2908, 2009

18. Cairncross JG, Macdonald DR, Pexman JH, et al: Steroid-induced CT changes in patients with recurrent malignant glioma. Neurology 38:724-726, 1988

19. Watling CJ, Lee DH, Macdonald DR, et al: Corticosteroid-induced magnetic resonance imaging changes in patients with recurrent malignant glioma. J Clin Oncol 12:1886-1889, 1994

20. Henegar MM, Moran CJ, Silbergeld DL: Early postoperative magnetic resonance imaging following nonneoplastic cortical resection. J Neurosurg 84:174-179, 1996

21. Kumar AJ, Leeds NE, Fuller GN, et al: Malignant gliomas: MR imaging spectrum of radiation therapy- and chemotherapy-induced necrosis of the brain after treatment. Radiology 217:377-384, 2000

22. Ulmer S, Braga TA, Barker FG 2nd, et al: Clinical and radiographic features of peritumoral infarction following resection of glioblastoma. Neurology 67:1668-1670, 2006

23. Finn MA, Blumenthal DT, Salzman KL, et al: Transient postictal MRI changes in patients with brain tumors may mimic disease progression. Surg Neurol 67:246-250, 2007

24. Stupp R, Mason WP, van den Bent MJ, et al: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-996, 2005

25. Brem SS, Bierman PJ, Black P, et al: Central nervous system cancers. J Natl Compr Canc Netw 6:456-504, 2008

26. de Wit MC, de Bruin HG, Eijkenboom W, et al: Immediate post-radiotherapy changes in malignant glioma can mimic tumor progression. Neurology 63:535-537, 2004

27. Brandsma D, Stalpers L, Taal W, et al: Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. Lancet Oncol 9:453-461, 2008

28. Chamberlain MC, Glantz MJ, Chalmers L, et al: Early necrosis following concurrent Temodar and radiotherapy in patients with glioblastoma. J Neurooncol 82:81-83, 2007

29. Taal W, Brandsma D, de Bruin HG, et al: Incidence of early pseudo-progression in a cohort of malignant glioma patients treated with chemoirradiation with temozolomide. Cancer 113:405-410, 2008

30. Brandes AA, Franceschi E, Tosoni A, et al: MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol 26:2192-2197, 2008

31. Cairncross JG, Pexman JH, Rathbone MP, et al: Postoperative contrast enhancement in patients with brain tumor. Ann Neurol 17:570-572, 1985

32. Sato N, Bronen RA, Sze G, et al: Postoperative changes in the brain: MR imaging findings in patients without neoplasms. Radiology 204:839-846, 1997

33. Cairncross JG, Pexman JH, Rathbone MP: Post-surgical contrast enhancement mimicking residual brain tumour. Can J Neurol Sci 12:75, 1985

34. Smith JS, Cha S, Mayo MC, et al: Serial diffusion-weighted magnetic resonance imaging in cases of glioma: Distinguishing tumor recurrence from postresection injury. J Neurosurg 103:428-438, 2005

35. Matheus MG, Castillo M, Ewend M, et al: CT and MR imaging after placement of the GliaSite radiation therapy system to treat brain tumor: Initial experience. Am J Neuroradiol 25:1211-1217, 2004

36. Parney IF, Kunwar S, McDermott M, et al: Neuroradiographic changes following convection-enhanced delivery of the recombinant cytotoxin interleukin 13-PE38QQR for recurrent malignant glioma. J Neurosurg 102:267-275, 2005

37. Floeth FW, Aulich A, Langen KJ, et al: MR imaging and single-photon emission CT findings after gene therapy for human glioblastoma. Am J Neuroradiol 22:1517-1527, 2001

38. Ross DA, Sandler HM, Balter JM, et al: Imaging changes after stereotactic radiosurgery of primary and secondary malignant brain tumors. J Neurooncol 56:175-181, 2002

39. Young GS: Advanced MRI of adult brain tumors. Neurol Clin 25:947-973, 2007

40. Chen W: Clinical applications of PET in brain tumors. J Nucl Med 48:1468-1481, 2007

41. Soares DP, Law M: Magnetic resonance spectroscopy of the brain: Review of metabolites and clinical applications. Clin Radiol 64:12-21, 2009

42. Sibtain NA, Howe FA, Saunders DE: The clinical value of proton magnetic resonance spectroscopy in adult brain tumours. Clin Radiol 62:109-119, 2007

43. Batchelor T, Sorensen A, di Tomaso E, et al: AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 11:83-95, 2007

44. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, et al: Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 25:4722-4729, 2007

45. Friedman HS, Prados MD, Wen PY, et al: Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 27:4733-4740, 2009

46. Kreisl TN, Kim L, Moore K, et al: Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol 27:740-745, 2009

47. Norden AD, Drappatz J, Muzikansky A, et al: An exploratory survival analysis of anti-angiogenic therapy for recurrent malignant glioma. J Neurooncol 92:149-155, 2009

48. Sorensen AG, Batchelor TT, Zhang WT, et al: A "vascular normalization index" as potential mechanistic biomarker to predict survival after a single dose of cediranib in recurrent glioblastoma patients. Cancer Res 69:5296-5300, 2009

49. Norden AD, Young GS, Setayesh K, et al: Bevacizumab for recurrent malignant gliomas: Efficacy, toxicity, and patterns of recurrence. Neurology 70:779-787, 2008

50. Narayana A, Raza S, Golfinos JG, et al: Bevacizumab therapy in recurrent high grade glioma: Impact on local control and survival. J Clin Oncol 26:691s, 2008 (suppl; abstr 13000)

51. Norden AD, Drappatz J, Wen PY: Novel anti-angiogenic therapies for malignant gliomas. Lancet Neurol 7:1152-1160, 2008

52. Rubenstein J, Kim J, Ozawa T, et al: Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. Neoplasia 2:306-314, 2000

53. Paez-Ribes M, Allen E, Hudock J, et al: Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 15:220-231, 2009

54. Bergers G, Hanahan D: Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 8:592-603, 2008

55. Chawla S, Poptani H, Melhem ER: Anatomic, physiologic and metabolic imaging in neuro-oncology. Cancer Treat Res 143:3-42, 2008

56. Ullrich RT, Kracht LW, Jacobs AH: Neuroimaging in patients with gliomas. Semin Neurol 28: 484-494, 2008

Appendix 7. Karnofsky Performance Status Scale

Karnofsky Performance Status Scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self, unable to carry on normal activity or to do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization indicated Death not imminent
20	Very sick, hospitalization indicated Death not imminent
10	Moribund, fatal processes progressing rapidly
0	Dead

Appendix 8. European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire, with a brain-cancer module (EORTC QLQ-C30/BN20)

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ____

Your birthdate (Day, Month, Year): ____

Today's date (Day, Month, Year): 31 ____

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

ENGLISH

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

ENGLISH

EORTC QLQ - BN20

Patients sometimes report that they have the following symptoms. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:		Not at All	A Little	Quite a Bit	Very Much
31.	Did you feel uncertain about the future?	1	2	3	4
32.	Did you feel you had setbacks in your condition?	1	2	3	4
33.	Were you concerned about disruption of family life?	1	2	3	4
34.	Did you have headaches?	1	2	3	4
35.	Did your outlook on the future worsen?	1	2	3	4
36.	Did you have double vision?	1	2	3	4
37.	Was your vision blurred?	1	2	3	4
38.	Did you have difficulty reading because of your vision?	1	2	3	4
39.	Did you have seizures?	1	2	3	4
40.	Did you have weakness on one side of your body?	1	2	3	4
41.	Did you have trouble finding the right words to express yourself?	1	2	3	4
42.	Did you have difficulty speaking?	1	2	3	4
43.	Did you have trouble communicating your thoughts?	1	2	3	4
44.	Did you feel drowsy during the daytime?	1	2	3	4
45.	Did you have trouble with your coordination?	1	2	3	4
46.	Did hair loss bother you?	1	2	3	4
47.	Did itching of your skin bother you?	1	2	3	4
48.	Did you have weakness of both legs?	1	2	3	4
49.	Did you feel unsteady on your feet?	1	2	3	4
50.	Did you have trouble controlling your bladder?	1	2	3	4

Appendix 9. Protocol Amendment Changes

Refer to Summary of Changes to Protocol Document 02 August 2018

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CLINICAL STUDY PROTOCOL

Protocol Title:	A Phase 1/2 Dose-escalation of USL311 as Single Agent and in Combination with Lomustine (CCNU) in Subjects with Advanced Solid Tumors, with Subsequent Single Agent and Combination Phase 2 Cohorts for Subjects with Relapsed/Recurrent Glioblastoma Multiforme (GBM)
Protocol Number:	P311-201
Protocol Version:	Amendment 4
Issue Date:	02 Aug 2018

Rationale for Amendment:

In Protocol Amendment #3, in order to manage a potential USL311-related increase in the QT interval, which was transient, resolved post-end of infusion, and exhibited an apparent correlation with plasma USL311 concentrations (i.e., C_{max}), the duration of intravenous (IV) infusion was increased from 120 minutes (2 hours) to 240 minutes (4 hours). Dosing with the 4-hour infusion began at 180 mg/m^2 and progressed to 250 mg/m^2. After a total 13 subjects, including the original 8 subjects that prompted Amendment #3, were evaluable (i.e., had completed Cycle 1 dosing, at minimum), the Dose Escalation Committee halted dose escalation, although no DLTs had occurred, due to continuing dose-related increases in QTcF prolongation that was not ameliorated by increasing the infusion duration.

The protocol is being amended to change to an oral formulation of USL311. Daily oral dosing is anticipated to mitigate the concentration-related QT effect, compared to IV bolus weekly dosing. The selected 40 mg daily oral starting dose is comparable to the 30 mg daily starting dose based on 1/6 HNSTD in dogs (5 mg/kg/day oral daily dose) and is lower than the ~60 mg daily dose calculated based on the maximum IV dose tested in humans (250 mg/m2 weekly, divided into daily doses). USL311 plasma pharmacokinetics are dose-proportional and minimal to no plasma accumulation is expected for USL311 and its main metabolites after daily oral dosing. A fixed-

dosing approach is considered to be appropriate for future studies given that USL311 pharmacokinetics are not influenced by body size.

An additional Phase 1 dose-escalation cohort of subjects with advanced solid tumors has been added (designated Part 1b) to determine the oral USL311 maximum tolerated dose (MTD), the recommended Phase 2 dose (RP2D), and starting dose for Phase 1 combination with oral lomustine.

Summary of key changes

Change	Rationale	Key sections affected
Sponsor information changed to Proximagen LLC	Divestiture of Proximagen subsidiary from Upsher-Smith Laboratories, LLC	Title page; SAE reporting information contact information
Rationale for oral starting dose	Toxicology study in dogs with oral formulation	1.2.4 Toxicology and Safety Pharmacology; 6.1.2.1 Phase 1
Updated clinical study findings	Experience available on 13 subjects	1.3 Clinical Study Findings for USL311;
Addition of Part 1b (oral) cohort	Change to oral dose form for Part 1b and all subsequent cohorts, with accompanying changes in schedule of assessments, including PK and PD	Schedule of Visits and Assessments Table 2 and 9; 3.2.2 Part 1b -Dose-escalation in Advanced Solid Tumors of Single Agent Oral USL311; 3.2.4 Dose-escalation Strategy; 3.2.5 Dose Limiting Toxicities; 7.2.2 Part 1b Treatment; 8.6.1 Whole Blood and Plasma Sample Collection; 8.7.3 Collection of Other Biomarkers; Appendix 1b Adaptive Design Rpeort for the Part 1b Dose-Escalation of Oral USL311 in Subjects with Advanced Solid Tumors
Increase in subject number	Addition of Phase 1 Part 1b oral dose cohort	5.1 Number of subjects
Addition of allergy/hypersensitivity to components of USL311 formulation to Exclusion 11 and addition of Exclusion 17 regarding conditions that may have a significant effect on USL311 absorption	To cover oral formulation excipients and oral dosing	5.2.2 Exclusion Criteria;
Addition of concomitant medication and dietary restrictions	To cover oral dosing	5.3.2 Prohibited Medications; 5.4 Dietary and Other Protocol Restrictions;
Addition of oral dose and dosing information	To cover oral dosing	6.1 Study Treatment Administration and Doses; 6.1.1.4 USL311 Oral Administration; 6.1.5.1 USL311 Dose Modification; 6.1.7 Treatment Compliance; 6.4 Study Drug Identification and Supply; 6.6 Handling, Storage and Accountability; 6.6.1 Dispensing of Study Drug

Removal of SDF-1 from Part 1b and beyond	Validated assay not available	8.7.3 Collection of Other Biomarkers
Changes in assessments throughout	To cover oral dosing	Multiple
Clarifications and typographical corrections	Clarify prior text, make text consistent, and correct errors in prior protocol. Format and minor changes are not tracked.	Multiple